Characterisation and optimisation of the flavour of health-promoting, plant-derived bitterants in functional beverages.

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ABSTRACT

Flavour is a combination of taste, odour, and chemesthetic sensations. Close associations exist between these sensory modalities, and thus, the overall flavour of a food or beverage product can change when the intensity of one or more of these sensations is altered. Strategies to modify flavour are often utilized by the food industry, and are central to the engineering of new and reformulated products. For functional food and beverages, flavour modification is particularly important, as fortifying agents can elicit high levels of less than desirable sensations, such as bitterness and astringency. The application of various flavour modifying strategies can decrease the perceived intensity of these sensations, and in turn, improve the sensory profile of the product. This collection of studies describes the sensory characteristics of experimental functional beverages fortified with *trans*-resveratrol, (+)-catechin, and/or caffeine, and examines the impact of novel flavour modifying strategies on the perceived flavour of these beverages. In the first study, results demonstrate that the flavour profile of Cabernet Sauvignon wines fortified with 20 mg/L and 200 mg/L of *trans*-resveratrol is not perceived as different compared to control wine (0 mg/L). However, Riesling wine fortified with 200 mg/L is perceived as significantly higher in bitterness compared to 20 mg/L and control. For some functional food formulations, alternative strategies for flavour modification are needed. Traditional methods, such as the addition of sucrose and sodium chloride, may decrease the perceived 'healthiness' of a product, and thus, may be sub-optimal. In a second study, high and low concentrations of five different bitter inhibiting compounds - 'bitter blockers' - (ß-cyclodextrin, homoeridictyol sodium salt, carboxymethylcellulose - low viscosity, zinc sulfate, magnesium sulfate) were tested for their efficacy towards decreasing the bitterness of high and low concentrations of caffeine and (+)-catechin - two health-relevant, plant-derived bitterants. ß-cyclodextrin and homoeridictyol

sodium salt were the most effective blockers at decreasing (+)-catechin and caffeine, respectively. In addition to bitter blockers, additional flavour modifying strategies, either alone or in combination - may also be successful in functional food formulations. Both sucrose and rebaudioside A - a plant-derived sweetener - were effective at decreasing the bitterness of (+)-catechin. When added to (+)-catechin along with ß-cyclodextrin, both sweeteners provided the most effective decrease in bitterness compared to binary, ternary, or quaternary mixtures of (+)-catechin together with bitter blockers, sweeteners, and/or odourants. The perceived intensity of sensations elicited by sweeteners and odourants was not affected by the addition of bitter blockers, and thus, their impact within these complex matrices is minimal. In addition, within-modal (taste-taste) compared to cross-modal (taste-odour) sensory interactions were more effective at decreasing the bitterness of (+)-catechin. Overall, results from these studies demonstrate that certain novel, alternative flavour modifying approaches may be successful towards lowering the bitterness and astringency elicited by (+)-catechin and caffeine in aqueous solutions.

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Chapter 1 - INTRODUCTION

A functional food can be defined as a product that 'is similar in appearance to, or may be, a conventional food, is consumed as part of a usual diet, and is demonstrated to have physiological benefits and/or reduce the risk of chronic disease beyond basic nutritional functions' (Health Canada, 2002). Some examples are omega-3 fatty acid enriched eggs (Lawlor et al., 2010), vitamin D fortified orange juice (Tangpricha et al., 2003), and plant sterol enhanced margarine (Weststrate and Meijer, 1998). Functional food and beverages is the fastest growing sector in the food industry (Verbeke et al., 2005), and an increased consumer interest in the adoption of a healthy lifestyle and/or maintaining good health is a major factor in the success of these products (Boue et al., 2009).

Functional foods may be fortified with various bioactives, including natural, plant-based compounds. Plant-derived ingredients, such as polyphenols, are associated with a number of positive health effects, including protection against cardiovascular disease (Stangl et al., 2007), neurodegenerative disease (Mandel and Youdim, 2004) and some cancers (Ramos, 2008). While the addition of these compounds into functional food and beverage formulations can provide nutritional value, at certain concentrations, some functional ingredients can elicit undesirable levels of bitterness and astringency. Thus, a major aim for the functional food and beverage industry is to create products that provide effective bioactivity and an acceptable flavour profile.

The foci of this dissertation are twofold and include: an investigation into the flavour profile of some functional food products and model formulations, and an exploration of alternative flavour modifying strategies towards improving the flavour of some plant-based functional ingredients. The results and conclusions from this will assist in the development of new functional food and beverage formulations, as well as provide insight into new flavour

optimizing strategies for such products. Overall, the creation of novel, plant-based fortified products with an acceptable flavour may assist in the increased consumption of these foods, and thus, a possible improvement in the general health of the Canadian population.

Outline of Dissertation

This dissertation is presented as a series of chapters that have been prepared for publication in peer-reviewed, scientific journals. Thus, some repetition will appear between certain chapters.

Chapter 2

This chapter presents an overview of bitter taste perception, bitter functional ingredients, and current applications used to modify bitterness in the context of various foods and pharmaceuticals. While bitter taste is an important component of some foods, including beer, wine and chocolate, it is less than desirable in others. Traditional strategies for bitter modification include the addition of sweet, salty and umami eliciting compounds, as well as some textural ingredients. Alternative strategies, including the use of bitter blocking compounds, have been used in some model systems, and a review of these is reported. Overall, a critical assessment of all bitter modifying methods is presented in this chapter, along with an evaluation for use in functional food systems. This work was accepted in November 2010 for publication in the journal *Critical Reviews in Food Science and Nutrition* (Gaudette and Pickering, 2011a).

Chapter 3

This chapter is an investigation into the sensory profile and chemical characteristics of *trans*-resveratrol fortified wine - a functional beverage product. *trans*-Resveratrol is a polyphenol within grape skins and has been demonstrated to be associated with a variety of health-promoting properties. Through the process of vinification, *trans*-resveratrol becomes extracted into wine and thus, can be found in many red wines. However, due to a number of factors, the concentration of *trans*-resveratrol varies greatly between grape varieties and wine styles, and thus, fortification may be an attractive option for producers wanting to create a product that contains a consistent concentration of this compound. This is the first study to determine the sensory and chemical characteristics of *trans*-resveratrol fortified wine. Three concentration levels were tested in both Riesling and Cabernet Sauvignon (0 mg/L, 20 mg/L, and 200 mg/L). Over a 58-week period, sensory evaluations (difference testing and descriptive analysis), chemical analyses (pH, TA, SO_2, TEAC, HPLC) and spectrophotometric measurements were performed to evaluate the quality of the wines. This work was published in May 2011 in the *Australian Journal of Grape and Wine Research* (Gaudette & Pickering, 2011b).

Chapter 4

For some functional food formulations, flavour modification is needed in order to decrease the perceived intensity of some less than desirable sensations, including bitterness. Tradionally, bitter modification within the food industry is based on the addition of sucrose and sodium chloride - compounds that are not typically associated with 'healthiness,' and thus, are not optimal for functional food and beverages. Alternative applications, such as bitter blocking compounds, have been primarily used in pharmaceutical systems. Their use in functional food

formulations may be of value, however, little information is known about the capacity that many bitter blockers have on decreasing the bitterness of plant-based, functional ingredients. In this chapter, five different bitter blockers at high and low concentrations were assessed for their ability to decrease the bitterness of high and low concentrations of (+)-catechin and caffeine - two health-relevant, plant-derived bitterants. This work was accepted in October 2011 for publication in the *Journal of Functional Foods* (Gaudette & Pickering, 2011c).

Chapter 5

In addition to bitter blockers, other flavour modifying strategies may include the use of tastants and odourants to mask the perception of less than desirable sensations, such as bitterness and astringency. Caloric sweeteners that can decrease the intensity of these, such as sucrose, may not be optimal for some functional food formulations. Alternatively, and in conjunction with bitter blockers, plant-based sweeteners and sweet-associated odourants may be effective approaches towards bitter modification. This chapter explores the effectiveness of bitter blockers, sweeteners, and odourants individually, and in all possible combinations, on decreasing the bitterness of (+)-catechin. It was submitted to the *Journal of Food Science* in October 2011, and is currently in review (Gaudette and Pickering, 2011d).

Chapter 6

Within and cross-modal sensory interactions between taste-taste and taste-odour, respectively, play a major role in flavour perception. While the combined use of bitter blockers, sweeteners and odourants may be an approach for bitterness modification in some functional food formulations, the impact of bitter blockers and these sensory interactions on overall flavour profile is unclear. This chapter determines the relationship between these factors and measures

their overall effect on perceived flavour. Dr. Jeannine Delwiche was a collaborator in the data analyses for this chapter. She contributed insight into choosing sub-groups from the main dataset to allow for the most effective ANOVA analyses and testing of the main hypotheses. This chapter is in preparation for submission to the journal *Flavour* (Gaudette et al., 2011, in preparation).

Chapter 7

This chapter provides an overall discussion and conclusion of the dissertation and presents future research ideas and themes.

Chapter 2 - MODIFYING BITTERNESS IN FUNCTIONAL FOOD SYSTEMS

Nicole J. Gaudette & Gary J. Pickering

The candidate is the primary author and contributor to this chapter. Acknowledgement is given to Dr. Paul Zelisko for assistance with chemical structure editing. Dr. Gary Pickering has provided various edits throughout the development of this chapter.

This work is in press in the journal *Critical Reviews in Food Science and Nutrition.*

BITTER TASTE

Evolutionary role and importance in the diet

Humans and other mammals typically reject bitter tasting food - a trait that is thought to have arisen from an evolutionary adaptation to avoid the ingestion of potentially poisonous foods (Glendinning, 1994; Fischer et al., 2004), as nearly all naturally occurring environmental toxins taste bitter to humans (Glendinning, 1994). The dependence on this trait for survival has decreased due to the implementation of food safety measures for those living in industrialized nations (Mattes, 1994), and thus, we might expect that the strong aversion to bitter taste in humans would decline. In addition, over the past 2 million years, the consumption of meat in the human diet has increased, while the quantity of plant tissues has decreased. This may provide a rationale for the lack of selective constraint on human *TAS2R* genes (Wang et al., 2004) which are responsible for bitter taste perception. However, while the need to perceive bitterness as a means for survival is no longer a necessity, bitter taste remains an important modulator of ingestive behaviours in humans, including food and beverage choice (Drewnowski and Gomez-Carneros, 2000), with subsequent association with diet-related nutritional status and overall health.

It has been well documented that the average North American diet lacks adequate intake of fruits and vegetables (Statistics Canada, 2007), which may be associated with an increased risk of some diseases, including various cancers, diabetes, heart disease, and obesity (Ford & Mokdad, 2001; Riboli & Norat, 2003; He et al., 2004; Hung et al., 2004). Low consumption of some fruits and vegetables, including broccoli, Brussels sprouts, and cabbage, have been linked to their bitter taste (Tepper, 1998). However, not all bitter foods are disliked – dark chocolate,

red wine, and coffee are typically enjoyable for many consumers (Tepper, 1998) - highlighting that the relationship between taste perception and food choice is complex and sometimes unpredictable. Various environmental factors are believed to influence this relationship, in addition to differences between individuals in physiological mechanisms that underpin perception of bitterness.

Transduction pathway

Bitterness is perceived when bitter-eliciting compounds interact with receptors within foliate and circumvallate papillae on the lateral and posterior surfaces of the tongue, respectively (Hoon et al., 1999; Adler et al., 2000). Within these papillae are hundreds (in foliate) and thousands (in circumvallate) of taste buds that contain taste receptor cells (TRCs), which for bitter taste includes the seven transmembrane G-protein coupled receptor (GPCR) family, *TAS2Rs* (Bartoshuk, 1993; Chandrashekar et al., 2000, 2006; Gilbertson et al., 2000).

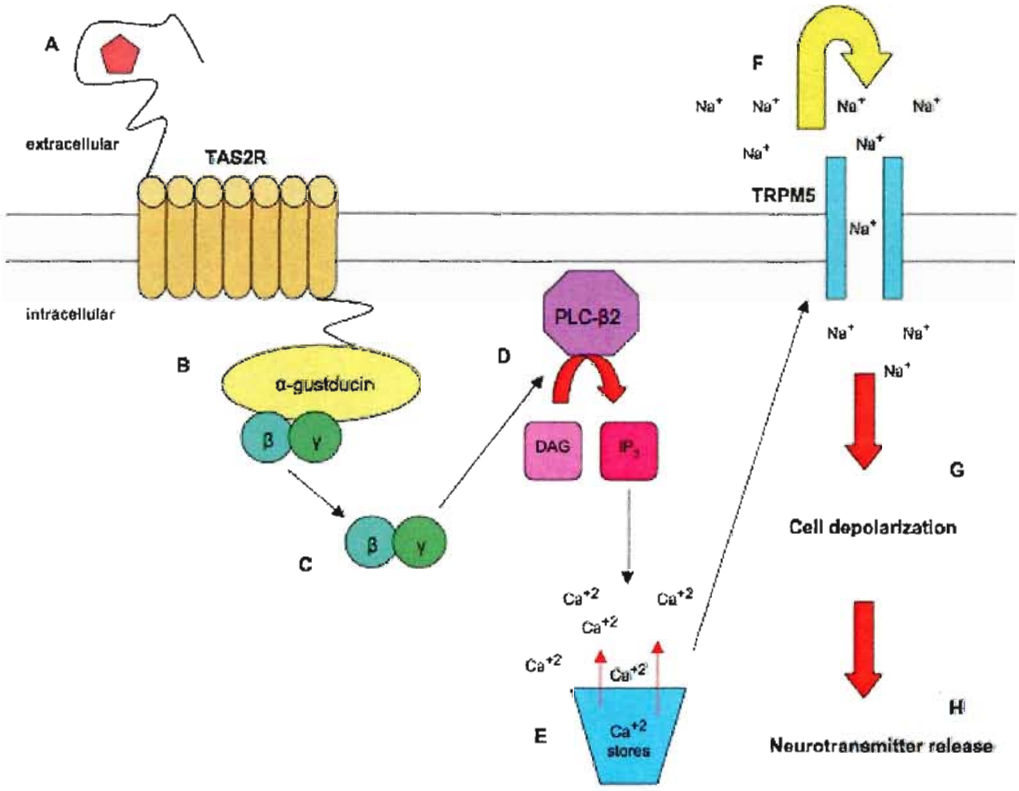
Bitter taste transduction occurs when bitter-eliciting compounds bind to receptors protruding through the taste pore on the apical surface of TRCs, generating a signal transduction cascade that results in activation of GPCRs and subsequent freeing of the $G_{\beta\gamma}$-subunit from the $G_{\alpha\beta\gamma}$heterotrimeric receptor (Figure 2.1). Release of the $G_{\beta\gamma}$-subunit activates phospholipase C (PLC-ß2), leading to the breakdown of phospholipids PIP_2, which produces intracellular inositol triphophate (IP_3) and diacylglycerol (DAG) (Roper, 2007). Increased levels of IP_3 stimulates IP_3 receptors and triggers the release of intracellular Ca^{+2} stores. From here, activation of TRPM5 occurs, resulting in the influx of Na^+ and subsequent receptor cell depolarization. Information is then conveyed to output cells where neurotransmitters are secreted (Roper, 2006), namely ATP

(Finger et al., 2005), although serotonin, norepinephrine, acetylcholine, glutamate and peptides have also been proposed (Roper, 2006). Neurotransmitters are then released from synaptic output cells onto gustatory afferent glossopharyngeal nerve fibres, generating an action potential that carries the chemical signal to information processing centers in the brain (Hoon et al., 1999; Margolskee, 2002; Gilbertson and Boughter, 2003; Roper, 2006, 2007). From here, the limbic system of the brain interprets the signal, and the quality and intensity of bitter taste is cognitively perceived.

Temporal perception

The temporal characteristics of bitterness are unique compared to the perception of other taste stimuli. Bitter taste requires more time to reach maximum intensity in the oral cavity, and the duration takes longer to return to baseline compared to sweet taste (Guinard et al., 1995). The perceived intensity of bitterness can also increase upon repeated ingestion (Guinard et al., 1986). This may be particularly important when consuming beverages such as red wine and beer, where the presence of polyphenolics (red wine) and isohumulones (beer) may lead to increasing bitterness of these beverages over the length of an ingestion session (Guinard et al., 1994; Noble, 1994). This may by exacerbated for products for which there is a culture of frequent sampling or sipping, such as can exist for wine and tea. Overall, the increase in bitterness of bitter tasting beverages over repeated ingestion may negatively impact their acceptance, as excessively bitter tasting foods are generally undesirable to the consumer (Drewnowski and Gomez-Carneros, 2000; Lesschaeve and Noble, 2005).

Figure 2.1 Schematic of bitter taste transduction mechanism. Bitter-eliciting compounds interact with and bind to TAS2R receptors (**A**). Binding of compounds activates GPCRs (**B**) and subsequently frees the βγ-subunit (**C**). Release of βγ-subunit activates phospholipase C (PLC-β2), resulting in the production of inositol triphosphate (IP_3) and diacylglycerol (DAG) (**D**). Increase in IP_3 triggers the release of intracellular calcium (Ca+2) stores (**E**). TRPM5 is activated, and an influx of sodium (Na+) results (**F**). Cell depolarization (**G**) and subsequent neurotransmitter release (**H**) relays a chemical signal to processing centers in the brain where bitterness is cognitively perceived. Adapted from Ley (2008) and Meyerhof (2005).



Moderators of bitter food behaviour

An individual's decision to consume specific foods and beverages is complex, and is influenced by multiple physiological, developmental and psychological processes, in addition to cultural and experiential factors. Bitter food choice and liking also involve numerous and inter-connected factors, however culture, environmental pressures, and genetic predisposition to orosensory sensations appear to be the primary influences.

Environmental experiences and cultural influences

From birth, humans are genetically predisposed to like sweetness, but to avoid bitterness and sourness. For example, when newborns are subjected to basic taste eliciting stimuli, gustofacial responses to sweet solutions result in a relaxed facial expression, and sometimes, licking and suckling movements (Steiner, 1977). In contrast, bitter tasting solutions evoke protrusion of the tongue and a depressed mouth angle, often followed by spitting or movements indicative of vomiting (Steiner, 1977). Although this predisposition exists, it does not suggest that bitter tasting foods are not liked later on in life. In fact, when encouraged by caregivers, repeated environmental exposure to disliked foods decreases the resistance to aversive tasting foods in children (Benton, 2004). However, the number of times an individual is exposed to a novel, bitter tasting food may be an important factor related to the overall liking of that food item. For instance, pleasantness ratings do not increase when a novel bitter melon drink is consumed 10 times over a 14 day period, whereas, an increase in rating does occur for a novel sweet jelly dessert (Mattes, 1994). Importantly, this does not infer that increased liking of novel, bitter foods cannot occur, but that bitter tasting foods may require a higher number of repeated

exposures to elevate liking compared to foods eliciting sweet taste (Mattes, 1994).

There is also evidence that acceptance of bitter tasting foods is affected by cultural factors. When preference tests of basic taste eliciting compounds were conducted on Indian laborers from the Karnataka region, differences were found compared to preference test results for Western populations (Moscowitz et al., 1975). Karnataka laborers rated citric acid increasingly pleasant as concentration increased, and rated low concentrations of quinine sulfate as exceptionally pleasant. In contrast, Western populations rate both of these tastes as unpleasant. The difference in liking of citric acid and quinine sulfate may be due to the heavy use of sour tasting foods in the diet of Karnataka laborers, including tamarind fruit (Moscowitz et al., 1975). Thus, genetic predispositions towards the dislike of certain tastes elicited by foods, especially bitter ones, may be modified by both repeated exposure and cultural experiences.

Genetic influences

While environmental experiences and cultural influences can influence the acceptance of bitter foods and beverages, an individual's genetic predisposition to the perception of bitterness can play an important role.

Differences in the ability to perceive bitter taste were first documented nearly 80 years ago (Fox, 1931, 1932), when the compound phenylthiocarbamide (PTC) was noted as being extremely bitter to some individuals, but to others, it elicited no taste at all. Since this initial finding, PTC, and now more commonly the chemically related 6-*n*-propylthiouracil (PROP) (Bartoshuk et al., 1994; Guo and Reed, 2001), have been used by researchers to investigate individual differences in taste perception (Bartoshuk et al., 1988, 1994, 1998).

The ability to perceive PROP has been associated with the taste intensity experienced when ingesting bitter foods and beverages, as well as their liking (Bartoshuk et al., 1994; Drewnowski et al., 1997; Tepper, 1998; Duffy & Bartoshuk, 2000; Lanier et al., 2005; Pickering et al., 2004; Bell and Tepper, 2006). Individual variation in PROP responsiveness is genetically influenced, with individuals categorized into one of three groups: supertasters (STs, perceive PROP intensely), medium tasters (MTs, perceive PROP moderately), or non-tasters (NTs, perceive PROP at a low intensity or not at all) (Bartoshuk et al., 1994). The gross variation that exists in the ability to perceive PROP is associated with the *TAS2R38* gene, which expresses two haplotypes (PAV and AVI) to generally represent the three PROP taste status (PTS) groups: PAV/PAV (STs), PAV/AVI (MTs), AVI/AVI (NTs) (Kim et al., 2003; Bufe et al., 2005). However, recent work by Hayes et al. (2008) suggests that PTS results from multiple factors, and not just the TAS2R38 genotype, including papillae density and perhaps additional receptors (Meyerhof et al., 2010).

The ability to perceive PROP has been associated in some studies to the liking and acceptance of various foods and beverages, especially those containing health-promoting compounds that are bitter in taste, such as polyphenolics in tea, chocolate and red wine, glucosinolates in cruciferous vegetables, flavonoids and limonoids in citrus fruit, and isoflavones in soy foods (Drewnowski and Rock, 1995; Drewnowski and Gomez-Carneros, 2000; Drewnowski et al., 2001). In young women, dietary preference for bitter tasting foods and beverages, including Brussels sprouts, cabbage, spinach and coffee, is associated with a greater responsiveness to PROP (Drewnowski et al., 1999), and pre-school aged children categorized as PROP tasters have lower preferences for raw broccoli compared to PROP non-tasters (Keller et al., 2002). In addition, NT children consumed more vegetables during a free-choice intake test,

especially bitter tasting ones (black olives, cucumbers, broccoli) compared to T children (Bell and Tepper, 2006).

Although there is evidence that PROP responsiveness is associated with food choice and liking, there are examples where this relationship has not been found. Keller et al. (2002) did not find a relationship between PROP responsiveness and cooked broccoli, orange juice, grapefruit-orange juice, milk chocolate or semisweet chocolate. Negative (for women) and positive (for men) correlations were found between PROP responsiveness and the acceptance of various sweet and fatty foods. No association for vegetables (eggplant, asparagus, spinach, etc.), cruciferous vegetables, or bitter tasting beverages was found for either sex (Duffy and Bartoshuk, 2000). When asked to choose the best liked food items, PROP taster and non-taster kindergarten and first-grade students did not differ when choosing spinach, raw and cooked broccoli. In addition, hedonic ratings for these foods did not differ (Anliker et al., 1991).

More recently, bitterness perception has been linked to another phenomenon believed to be under genetic control – thermal taste. When a small area of the tongue is heated and/or cooled, thermal tasters (TTs), who constitute approximately 20-50% of the population, perceive a phantom taste (Cruz and Green, 2000; Green and George, 2004; Bajec and Pickering, 2008). This phenotype has been proposed as a new marker of variation in oral sensation, as TTs also perceive a range of taste and some oral trigeminal sensations more intensively than thermal non-tasters (TnTs). Importantly, TTs rate the intensity of bitterants presented in aqueous solutions (Green and George, 2004; Bajec and Pickering, 2008) and the bitterness elicited by some wine (Pickering et al., 2010) and beer (Pickering et al. 2010, unpublished data) higher than TnTs.

Variation in TRPM5, a TRP superfamily cation channel with a role in the transduction of bitter, sweet and umami tastes (Zhang et al., 2003), has been proposed as the mechanism

underlying the thermal taste phenomenon (Talavera et al., 2005), although it does not adequately explain the heightened responsiveness of TTs to sour, salty, astringent or retronasally-presented stimuli reported in some studies (Green and George, 2004; Bajec and Pickering, 2008; Pickering et al., 2010). In contrast with the PROP literature, there has been only limited research to date on the implications of the heightened responsiveness to oral sensations in TTs for liking and consumption behaviours. Bajec and Pickering (2010) reported that TTs gave significantly lower liking scores to some groupings of bitter foods, while no differences in liking were found between TTs and TnTs for wine (Pickering et al., 2010) or beer (Pickering et al., 2010 unpublished data) of varying styles.

Overall, the relationship between PROP responsiveness, thermal taste status and food behaviours are not always predictable. However, these phenotypes may play significant roles in the food choices that one makes on a daily basis, and need to be considered when accounting for the acceptance and consumption of healthy, bitter tasting foods, and diet-related health status (Tepper, 1998, 2008; Garcia-Bailo et al., 2009).

FUNCTIONAL FOODS

Functional foods is the fastest growing sector in the food industry (Verbeke, 2005), yet, many of these products contain ingredients that elicit less than desirable tastes and flavours that can impact the product's overall consumer acceptability (Kolanowski et al., 2001; Dervisoglu et al., 2005; Mohamed et al., 2006). While the presence of health claims (Tuorila and Cardello, 2002), frequency in use of a related, conventional product (Luckow and Delahunty, 2004), gender (Verbeke, 2005), and age (Bech-Larsen et al., 2001) play a role in individual choices regarding functional foods, a significant factor in their acceptance is their overall taste and

flavour (Verbeke, 2006).

Bitter tasting functional ingredients

The increased consumer interest in functional foods in recent years has helped to spawn the development of new functional ingredients and products. For example, products such as broccoli sprout beverages are on the market, and broccoli cultivars with increased levels of glucosinolates have been bred (Faulkner et al., 1998; Sarikamis et al., 2006). In addition, polyphenolics represent an increasing proportion of the new additives and are found in a range of products, from flavonoid-enriched chocolate bars to *trans*-resveratrol fortified wine (Table 2.1). However, the potential for these new functional ingredients to impart undesirable bitter and mouthfeel characteristics is a limitation to greater consumer acceptance and market success.

Polyphenolics as fortifying functional ingredients

Polyhenolics are a large class of compounds with significant anti-oxidative capacity (Waterhouse, 2002) (Figure 2.2). They can be found in a variety of plant species, including grapes, and in their by-products, such as red wine (Waterhouse, 2002). Wine polyphenolics include 2 main families of phenol-containing compounds – the flavonoids and the non-flavonoids. The non-flavanoids include hydroxycinnamates, benzoic acids, hydrolysable

Table 2.1 Some commercial functional food products containing endogenous or added bitter-eliciting functional ingredients.

Product	Active functional ingredient	Company
Broccoli sprout juice	Glucosinolates (sulforaphane)	Garden Gate Farms Ontario, Canada
Green tea energy drink Enviga™	Green tea phenolics [a]	The Coca-Cola Company Georgia, U.S.A.
Catechin enriched green tea powder	Green tea phenolics [a]	Shizuoka Tea Japan
Matcha green tea yogurt	Green tea phenolics [a]	Trader Joe's® California, U.S.A.
Yogurt with green tea extract Silhouette 0[+]®	Green tea phenolics [a]	Danone, Incorporated Quebec, Canada
Chocolate bar CocoaVia®	Cocoa flavonoids [b]	Mars, Incorporated Virginia, U.S.A.
Chocolate dietary supplement drink CirkuHealth™	Cocoa flavonoids [b]	Mars, Incorporated Virginia, U.S.A.
Water Vitaminwater ™ – "sync" berry-cherry	Berry phenolics	Glaceau Vitaminwater® New York, U.S.A.
Grape skin powder Cabernet Grape Powder - used to fortify breads	Grape phenolics [c]	Vinifera For Life Canada™ Ontario, Canada
Sports drink Xilarate ™ Sports Power Fluid	Grape seed extract [c]	Xilarate Ontario, Canada
Polyphenolic enhanced wine	Resveratrol	The Wine Doctor Resveratrol Enhanced Wines NSW, Australia
Nutrition bar soy protein bar	Soy isoflavones	GeniSoy® Oaklahoma, U.S.A.

[a] can include epigallocatechin gallate and (+)-catechin (Higdon & Frei, 2003)
[b] can include epicatechin and procyanidin B2 (Lameula-Raventós et al., 2005)
[c] can include *trans*-resveratrol, quercetin, (+)-catechin, and (-)-epicatechin (Waterhouse, 2002)

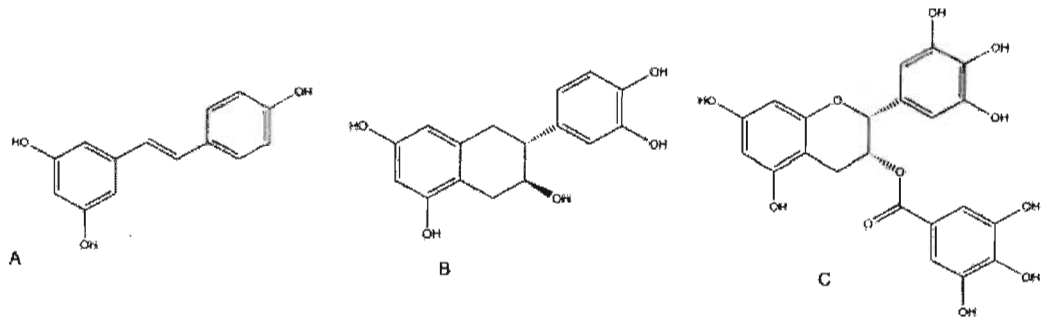


Figure 2.2 Chemical structure of 3 phenolics: *trans*-resveratrol (A), (+)-catechin (B), and epigallocatechin gallate (C).

tannins, and stilbenes (*trans*-resveratrol, *trans*-piceid), while the flavonoids include flavon-3-ols [e.g., (+)-catechin, (-)-epicatechin], flavonols (e.g., quercetin, kaempferol, myricetin), and anthocyanins (e.g., malvidin 3-glucoside, delphinidin 3-glucosode) (Waterhouse, 2002; Clarke and Bakker, 2004).

The interest in polyphenolics as functional ingredients is due to their myriad of health-promoting properties, which include protection against certain cancers and cardiovascular disease (Savouret and Quesne, 2002; Delmas et al., 2005). For instance, *trans*-resveratrol (3,4,5' trihydroxy-*trans*-stilbene), a polyphenolic primarily found in grapes (LeBlanc et al., 2006) and wine (Siemann and Creasy, 1992), has been shown to help protect against colon and breast cancers (Jang et al., 1997; Schneider et al., 2000; Nakagawa et al., 2001) and diabetes (Palsamy and Subramanian, 2008). There is also evidence that it provides a cardioprotective effect via inhibition of LDL oxidation, increased endothelial nitric oxide production, and inhibition of platelet aggregation (Frankel and Kanner, 1993; Hung et al., 2000; Wallerath et al, 2002; Wang et al., 2002). In addition, it has been shown to significantly decrease the negative effects of a

high-caloric diet (Baur et al., 2006).

Polyphenolic fortified functional foods are of interest and value to consumers, primarily due to their perceived health benefits (Barreiro-Hurlé et al., 2008). However, the potential success of these products may be limited by excessive bitterness and astringency - oral sensations known to be elicited by some polyphenolics (Robichaud & Noble, 1990; Thorngate & Noble, 1995; Kallithraka et al., 1997; Kielhorn & Thorngate, 1999; Peleg et al., 1999; Brossaud et al., 2001; Vidal et al., 2004).

Polyphenolics have been extracted from plant material and used to fortify various products (Shi et al., 2005). Fortification of meats with citrus by-product containing flavonoids produce an acceptable product (Fernández-López et al., 2004), but when resveratrol is added to Brehwurst, the product is less flavourful and elicits lower levels of meat aroma intensity (Nitsch, 2005). Polyphenolics, including resveratrol, quercetin, and fisetin have also been used as fortifying agents in yogurt, although the sensory characteristics of this product have not been reported (Ramarao and Alla, 2008). Quercetin has been assessed for its water solubility, with the goal of supplementing it into a variety of beverages including fruit juice and wine (Howard, 2003). Additionally, when apple and grape seed polyphenolics are added to milk, typical milk flavour characteristics are suppressed, and a high level of bitterness is elicited, particularly due to apple seed polyphenolics (Axten et al., 2008).

In an effort to increase the consumption of functional foods, and in turn, improve the overall health status of the population, it is attractive to fortify various foods with health-promoting compounds including polyphenolics. However, some polyphenolics can elicit sensations that may impact the overall sensory profile of a product. For instance, (+)-catechin and (-)-epicatechin elicit bitterness and astringency (Peleg et al., 1999), while dihydrochalcones

elicit sweetness (Gent and Bartoshuk, 1983). In addition, anthocyanins and some flavanones elicit very little or no taste at all (Ley et al., 2002; Vidal et al., 2004).

The oral perception of some polyphenolics depends upon their degree of polymerization. Monomer (+)-catechin and (-)-epicatechin units are perceived as more bitter than astringent, while the intensity of their dimers and trimers are perceived equal and higher in astringency compared to bitterness, respectively (Peleg et al., 2004).

Overall, due to the potential for undesirable sensations to be elicited by polyphenolics, strategies are needed to decrease the bitter taste and astringency often imparted by these functional ingredients.

MODIFYING BITTERNESS

The food industry currently implements several bitter masking and suppressing techniques to decrease the bitterness elicited by functional products. Traditional techniques include the use of sweet tasting compounds, salts, odourants, and textures to mask bitterness. The use of bitter inhibiting compounds has also been employed or proposed by the pharmaceutical and food industries. In this section, we review and evaluate the traditional modes of bitterness modification and their possible usage in the functional food industry.

Basic taste eliciting compounds

Sweet taste

The bitterness of caffeine is suppressed upon addition of sucrose, with increasing sucrose concentrations resulting in corresponding decreases in perceived bitterness intensity (Calviño et

al., 1990). Conversely, suppression of sweetness from sucrose increases upon elevating caffeine concentrations (Calviño et al., 1990). Although, bitterness and sweetness have the ability to suppress each other, the relationship is concentration dependent, as low concentrations of sweet tasting compounds will not always suppress low concentrations of bitterants (Keast and Breslin, 2002a).

Similarly, solutions of sucrose and quinine hydrochloride also demonstrate mutual suppression (Lawless, 1982, 1986). However, the suppression of bitterness by sucrose decreases and even leads to an increase in bitterness if a sucrose rinse is sampled prior to a quinine hydrochloride-sucrose mixture (Lawless, 1982). This demonstrates that prior taste adaptation to sucrose decreases the ability of a sweet stimulus to suppress bitterness. Complex interactions such as these likely occur frequently during everyday food consumption, making the goal of inhibiting bitterness challenging.

Non-nutritive sweeteners are intensely sweet, with aspartame and sucralose 200 and 500-750 times sweeter than sucrose, respectively (Wiet and Beyts, 1992). Sweeteners, such as aspartame and sucralose, can decrease the bitter taste of pharmaceuticals (Suzuki et al., 2004), including quinine (Nakamura et al., 2002). However, at high concentrations, these sweeteners can elicit a bitter aftertaste (Ott et al., 1991), which should be taken into consideration when creating new products.

Sweet tasting compounds, such as sucrose, decrease bitterness through a central cognitive effect (Kroeze and Bartoshuk, 1985). This occurs when the intensity of one taste component in a mixture is perceived as suppressed or enhanced independent of physical interactions that occur between tastants in the oral cavity (Keast and Breslin, 2002a). This differs from an oral peripheral physiological effect, where perceived taste intensity is dependent on such interactions

(Keast and Breslin, 2002a). Central cognitive effects have been demonstrated through split-tongue studies, where approximately the same level of bitterness suppression (31-37%) by sweetness occurs when either QHCl and sucrose are mixed and applied to the whole tongue, or when they are applied separately but simultaneously to each side of the tongue (Kroeze and Bartoshuk, 1985).

Sweet tasting compounds are widely known for their ability to significantly decrease bitterness. However, many traditional sweeteners, including sucrose, may be less attractive for functional foods because of their caloric content. Non-caloric artificial sweeteners also face challenges, as consumers may perceive them as 'unnatural' and synthetic. Thus, natural plant-derived sweeteners, or natural sweetness-enhancing compounds (Ley et al., 2008) with minimal side-tastes may be preferable alternatives.

Salty taste

Studies have shown the capacity of salts to suppress a number of bitterants (Breslin and Beauchamp, 1995, 1997; Keast and Breslin, 2002c; Keast et al., 2004). Interestingly, bitter suppression is predominately dependent on the presence of the sodium cation (Li^+ also decreases bitterness; Breslin and Beauchamp, 1995), as salts that do not contain Na^+ are unable to decrease bitterness (Breslin and Beauchamp, 1995). This general result was supported in a later study, where sodium was found to be the most effective cation at inhibiting a range of bitter oral pharmaceuticals (Keast and Breslin, 2002c).

In more complex sweet-bitter mixtures of sucrose and urea, the addition of sodium acetate decreases bitterness and increases sweetness (Breslin and Beauchamp, 1995). Bitterness

suppresses sweetness, and therefore, it has been hypothesized that sodium acetate releases this suppression and thus allows sweetness to be perceived more intensely (Breslin and Beachamp, 1995; Keast et al., 2001).

It is interesting to note that bitterness does not suppress saltiness unless saltiness is perceived to be of a low intensity (Breslin and Beauchamp, 1995). This may be due to a central cognitive effect, for low intensities of saltiness rather than sodium ion concentrations are dependent on this suppression (Breslin and Beauchamp, 1995). Contrary to this, it has been suggested that Na^+ containing salts inhibit bitterness via oral peripheral physiological interactions (Breslin and Beauchamp, 1995). Support for this comes from split-tongue studies (Kroeze and Bartoshuk, 1985), where it has been calculated that NaCl suppresses the bitterness of QHCl by approximately 22% and 69% through central cognitive and peripheral mechanisms, respectively.

The mechanism(s) responsible for the peripheral suppression of bitterness by sodium is unknown. Sodium has, however, been postulated to exert its effect by: a) moderating or modulating ion channels and/or pumps, b) stabilizing the cell membrane, c) blocking of *TAS2R*s, or d) interacting with second messenger systems within TRCs (Keast and Breslin, 2002c) (Figure 2.3).

Overall, while there is some suggestion that salt suppresses bitterness via a central cognitive effect, evidence for the role of oral physiological interactions - mainly from split-tongue studies - is stronger. However, the two mechanisms are not mutually exclusive, and it is possible that both play a part. Further research is needed to determine the underlying processes responsible for bitterness suppression via salts. Practically, the use of salts to decrease bitterness may have minimal application in functional foods, as there is considerable market pressure to

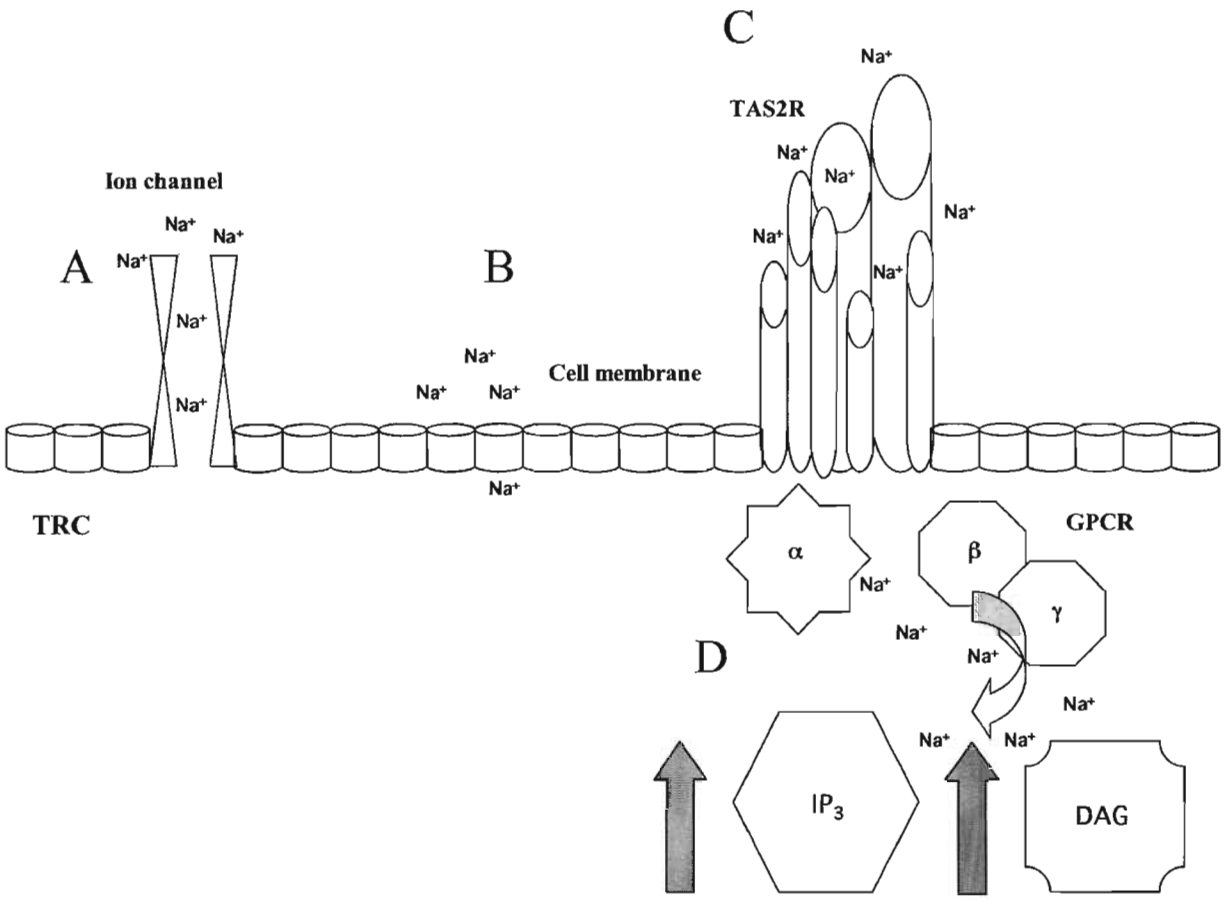


Figure 2.3. Schematic representation of the 4 current hypotheses regarding sodium's ability to suppress bitter taste perception: A) moderating or modulating ion channels and/or pumps, B) stabilizing the cell membrane, C) blocking of *TAS2R*s, or D) interacting with second messenger systems within TRCs (Keast and Breslin, 2002c).

TRC = taste receptor cell, TAS2R = general bitter taste receptor, Na^+ = sodium, IP_3 = inositol triphosphate, DAG = diacylglycerol, GPCR = G-protein coupled receptor.

lower the salt content of food, given its negative association with health status (Sacks et al., 2001).

Umami taste

Umami is generally defined as having a "savory" taste quality, and is elicited by glutamate containing anion salts, most notably, monosodium glutamate [MSG, (Löliger, 2000)]. In addition, umami taste can also be elicited by other anion salts, including adenosine monophosphate sodium and disodium salts (NaAMP, Na_2AMP) (Keast and Breslin, 2002c; Keast et al., 2004). Umami-eliciting compounds have been shown to impart a bitter inhibiting action on various bitterants. At suprathreshold concentrations, MSG inhibits the bitterness of quinine sulfate, although at threshold concentrations of MSG, no affect on the bitterness of quinine sulfate solutions is found (Kemp and Beauchamp, 1994).

In addition to MSG, NaAMP has been demonstrated to decrease the bitterness of a variety of oral pharmaceuticals, including pseudoephedrine, ranitidine, acetaminophen, quinine, and urea (Keast and Breslin, 2002c). For instance, the average intensity rating on the Labeled Magnitude Scale (LMS) of a 0.1 mM quinine hydrochloride (QHCl) solution is decreased from approximately 13 to 3 when NaAMP is added. Overall, in this study, 100 mM of NaAMP decreases bitter taste intensity by 65% across all oral pharmaceuticals. Similar results were found using MSG. However, other anion salts, including chlorine, salicylate, and gluconate, were generally not as effective as MSG and NaAMP at decreasing the bitterness of the pharmaceuticals examined.

In another study, 50 mM Na_2AMP decreased the bitterness of sixteen solutions consisting of single bitterants, double the concentration of single bitterants, or binary mixtures of bitterants (Keast et al., 2004). Overall, Na_2AMP significantly decreased the average bitterness of all binary mixtures (pooled), as rated using the generalized LMS (gLMS), by and average of 40%. Both MSG and the combination of MSG and inositol monophosphate (IMP), have similar bitter inhibiting capacity.

Of interest, 100 mM MSG and 20 mM MSG + 2.4 mM IMP were perceived to have higher umami taste intensities compared to 20 mM MSG. Yet, overall, these 3 mixtures did not differ in their ability to suppress bitterness (Keast et al., 2004). This suggests that the bitter inhibiting action of glutamate may be due to an oral peripheral rather than a central cognitive effect (Keast and Breslin, 2002a).

The mechanism by which NaAMP/Na_2AMP decreases bitterness is not known. However, as the transduction pathway is likely similar to MSG (Keast and Breslin, 2002c), AMP sodium salts may also inhibit bitterness due to an oral peripheral effect (Keast and Breslin, 2002a). In addition, bitterness masking due to the activity of Na^+ would be significant, and thus it is possible that AMP has only a minimal role in reducing bitterness.

While MSG imparts a savory taste (Löliger, 2000), AMP sodium salts elicit both savory and sour tastes (Keast and Breslin, 2002c), and thus, at concentrations examined thus far, these compounds may by less preferable for addition to functional foods as bitter inhibitors. Determination of dose-response functions using lower MSG and AMP sodium salt concentrations would be of value in establishing optimal concentrations for bitter inhibition while imparting minimal extraneous tastes. There is some suggestion that MSG is associated with negative health effects (Nakanishi et al., 2008), which, if substantiated, would limit its use

in functional foods. However, it has also been concluded that the use of glutamate salts as a food additive is safe for the general human population (Beyreuther et al., 2007).

Texture

There is some evidence that textural cues from increasing viscosity of aqueous solutions may decrease bitterness intensity, although, exceptions to this finding have also been reported. Increased cellulose gum concentrations (1-1000 cps) result in lower intensity ratings for quinine sulfate (0.03125×10^{-3} - 1.0×10^{-3}M) (Moskowitz and Arabie 1970). The bitterness of caffeine is also decreased by approximately 60% when stimuli are presented in a gelatin matrix compared to water (Calviño et al., 1993). Of interest, this study also demonstrated a trend of decreased bitter intensity in a carboxymethyl cellulose (CMC) matrix compared to water.

Several different hydrocolloids [hydroxypropyl cellulose (HPC), low viscosity CMC (CMC-L), medium viscosity CMC (CMC-M), sodium alginate (SA), and xanthan (X)] have been tested for their influence on bitterness (Pangborn et al., 1973). Of these, CMC-L, SA and X produce the largest reduction in caffeine bitterness ($0.46 - 3.7 \times 10^{-3}$M) (Pangborn et al., 1973). In a similar study, addition of these same hydrocolloids at increasing concentrations to bitter beverages, lead to significant decreases in coffee bitterness, especially for CMC-L (approximately 54% reduction) (Pangborn et al., 1979).

Of interest, CMC-M does not significantly alter the taste intensity of caffeine (Pangborn et al., 1973), or the bitterness of grape seed tannin (Smith et al., 1996). However, CMC-M does significantly decrease the bitterness of caffeine and coffee when a series of increasing concentrations of CMC-M are used (Pangborn et al., 1973, 1979). Thus, inhibition of bitterness depends on both the concentration of hydrocolloid and type of bitterant used.

It is interesting to note that in Moskowitz & Arabie (1970), panelists often reported that low concentrations of quinine sulfate (below 0.03125 x 10^{-3}M) were undetectable in solutions of high viscosity (range tested was 1 - 1000 cps). This suggests that blocking of access to TCR binding sites imparted by viscous compounds is particularly effective at low concentrations of bitterants.

The use of texture-modifying compounds to reduce bitterness may be particularly attractive with functional beverages. Natural, plant based gums decrease bitterness, while providing a source of dietary fibre, increasing the nutritional value of the product. However, optimization of gum type and concentration to the functional ingredient would be necessary, as, for instance, CMC-M does not affect the bitterness elicited by grape seed polyphenolics (Smith et al., 1996).

Odourants

When an odourant is added to a basic taste solution, the intensity of the basic taste has the potential to be enhanced or suppressed (Delwiche, 2004). Odour induced taste enhancement is more likely to occur when an odour and taste are cognitively associated with each other. For example, when strawberry odour is combined with a sucrose solution, the perceived intensity of sweetness is higher than that of sucrose alone. Pairing strawberry odour with a sodium chloride solution does not increase perceived saltiness (Frank and Byram, 1988). In addition, peanut butter odour does not increase the sweetness of sucrose, providing evidence that taste enhancement is both odour and taste dependent (Frank and Byram, 1988).

However, some research has suggested that odour-taste interactions can result in cross-modal summation independent of odour-taste agreement. In Delwiche and Heffelfinger (2005), a sweet-pineapple (aspartame/acesulfame potassium) pairing was chosen as a congruent

association, and a brothy-pineapple (MSG) pairing was chosen as an incongruent association. Following threshold determination of tastants and odourant, half-threshold concentrations of each odour-taste pair were presented. Subthreshold concentrations of both pairs, when presented against a water blank, resulted in percent correct responses greater than chance.

Although studies have investigated the effect of odourants on the perception of sweet, salty, sour, and umami tastes, few have explored the association with bitterness. Coffee and chocolate aromas were found to increase the bitterness of caffeine in fat free milk by 17% and 32%, respectively (Keast, 2008), and cocoa flavouring significantly increased the bitterness of cocoa beverages compared to control (cocoa beverage without flavouring) (Labbe et al., 2006). These results are not surprising, as bitterness is cognitively associated with these aromas and flavours. However, in the latter study, vanilla flavouring, which is congruent with sweet taste, increased the perception of sweetness but did not affect bitterness ratings. Additionally, the addition of vanilla flavouring to an unfamiliar bitter milk beverage increased perceived bitterness perception, suggesting that previous food experiences and/or food neophobia should be considered when developing optimal strategies for modifying bitterness with odourants and flavourings.

Central cognitive processing and the summation of aroma and taste to create flavour perception have been examined via fMRI studies, with the insula, orbitofrontal cortex, and anterior cingulated cortex implicated in flavour processing (Small et al., 2004). Of particular interest, neural activity in some parts of the brain is greater during the presentation of congruent odour-taste pairings, compared to when odour and taste are presented separately.

Overall, it is clear that further research is needed to determine the precise role that odour plays in bitterness perception. Little is known on whether incongruent odours decrease bitterness,

Table 2.2. Characteristics of bitter blockers.

Bitter blocker	Product used in	Compound effective against	Mode of action	Advantages	Disadvantages	Citations
β-cyclodextrin	[1]citrus juice production	[2,3]naringin, limonin	[1]molecular encapsulation	[3]effective bitter inhibitor (↓ by 50%)	[4]ineffective for some bitterants (QHCl, caffeine) [4]may elicit sweet taste at higher concentrations	[1]Szejtli and Szente, 2005; [2]Shaw and Wilson, 1983; [3]Konno et al., 1982; [4]Toda et al., 1981
Riboflavin binding protein	NCU	Numerous bitterants, including [2]QHCl, naringin, caffeine, denatonium	[2]hydrophobic interaction with bitterant [2]competition for sites on *TAS2Rs*	[2]extremely effective bitter inhibitor (↓ by ~ 100%)	[1]inhibits sweet taste long production time	Maehashi et al., [1]2007, [2]2008
Flavanones	NCU	Numerous bitterants, including [1,2]caffeine, [2]quinine, denatonium benzoate, paracetamol	not currently known	[1,2]effective bitter inhibitor (↓ by ~ 40%) [2]natural, plant derived source	not known if it is effective for bitter functional ingredients	[1]Ley et al, 2002; [2]Ley et al, 2005
Phosphatidic acid-β-lactoglobulin (PA-LG)	NCU	[2,3]QHCl, [3]papavrine HCl, [3]L-leucine, [2]caffeine, [2]propranolol, [2]promethazine	[1]blocking of *TAS2Rs* (PA) [1]adsorption onto bitterant (PA)	[4]derived from food sources [2]does not inhibit other tastes	[4]expensive to produce, [4]difficult to preserve	[1]Nakamura et al., 2002; Katsuragi et al., [2]1995, [3]1996, [4]1997
Neodiosmin	NCU	[1]caffeine, [1]quinine sulfate, [2]limonin, [2]naringin	not currently known	[3]derived from natural plant source [1,2]decreases taste intensity of some bitterants in water (↓ by 80-300%) and orange juice (↓ by 53%)	expensive to source	[1]U.S. Patent No. 4,154,862, 1979; Guidagni et al., [2]1976; [3]Del Rio et al., 1992

Zinc salts	NCU	numerous bitterants, including [1]caffeine, [2]QHCl, [2]tetralone, [2]denatonium	[4]interaction with amino acids (serine, threonine) on TRCs	readily available [6]can increase nutritive value of food [2]potent inhibitor of some bitterants (↓ by 40% on avg; [4]↓ by 70% for QHCl)	[4]elicits astringency and umami [3,4,5]inhibits sweet taste	[1]Keast, 2008; [2]Keast and Breslin, 2005; [3]Keast et al, 2004, [4]Keast, 2003; [6]Salgueiro et al., 2002
Magnesium	NCU	[1]QHCl	not currently known	[1]effective inhibitor for some bitterants (↓ by ~ 52% for QHCl) [1]does not alter other tastes	[2]can elicit bitterness, sourness and saltiness at high concentrations	[1]Keast, 2003; [2]Delwiche et al., 2001
Fatty acids	fortifying agents in functional foods	[1]caffeine, [2]quinine sulfate, [2]leucine	[3]possible modulation of taste receptor cells [4]possible blocking of taste receptor cells	[7,8]valuable nutritive ingredient [1]increase taste threshold of caffeine [2,4,5]significant inhibitor of quinine (↓ by 22-40%)	[9]can impart negative sensory attributes at high concentrations [9]susceptible to oxidation	[1]Mattes, 2007; [2]Koriyama et al., 2002; [3]Gilbertson et al., 1997; [4]Lynch et al., 1993; [5]Valentová and Pokorný, 1998; [6]Kris-Etherton et al., 2003; Simopoulos, [8]1991, [7]1997; [9]Kolanowski et al., 1999

NCU = not currently used
TAS2Rs = a family of G-protein coupled receptors that mediate bitter taste
QHCL = quinine hydrochloride
HCl = hydrochloric adic
PA = phosphatidic acid
TRCs = taste receptor cells

or whether certain odourant-bitterant pairings are optimal in bitterness masking. Such research could ultimately lead to the development of new odourants and/or pairings able to decrease the bitterness of functional ingredients. Since odours do not negatively affect the nutritional properties of a product, they may be particularly attractive in the development of successful functional foods and beverages.

Bitter inhibiting compounds

In addition to traditional strategies currently used for bitter taste modification, several bitter inhibiting compounds, or 'bitter blockers', decrease bitter taste through complexation or encapsulation of bitterants, through interacting with bitter binding sites on taste receptor cells, or perhaps through interference with taste transduction mechanisms further downstream (Table 2.2).

While previous reviews have provided excellent and exhaustive considerations of bitter masking in the food and pharmaceutical industries (Roy, 1992; Ley, 2008; Mennella and Beauchamp, 2008; Sharma and Lewis, 2010), taste masking in functional foods poses a unique problem. Many of the traditional, bitter making approaches and ingredients used in other areas may decrease the 'healthiness' of functional foods. In addition, the primary goal can be different in these other industries. For instance, with pharmaceuticals the main objective is to decrease the bitterness of active ingredients to an acceptable/palatable level, whereas for functional foods, the overall sensory attributes of the product must be acceptable enough to encourage repeat consumer purchasing behaviour. Considering this, the following section describes the actions of the most common and promising bitter blockers, and evaluates their potential use within functional food formulations.

Cyclodextrins

Cyclodextrins (CD) are cyclic oligosaccharides that contain 6, 7, or 8 glucose units (α, β, or γ, respectively) attached by α-1-4 glycosidic linkages (Toda et al., 1981), resembling a donut-like or wreath-type structure (Szejtli and Szente, 2005) (Figure 2.4).

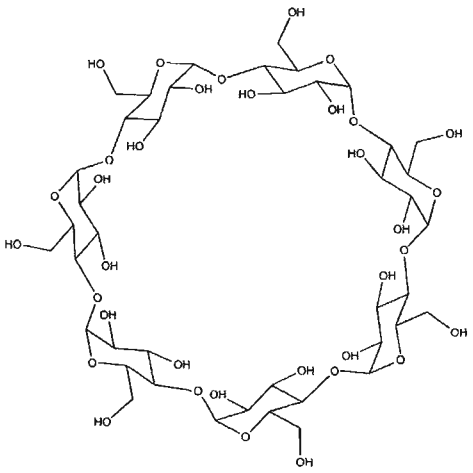


Figure 2.4. Chemical structure of β-cyclodextrin.

Cyclodextrins have multiple applications in food, including protection against the oxidative degradation of lipids, solubilization of food constituents including vitamins, stability of flavours, vitamins and lipids, controlled release of various food elements, and inhibition of unwanted odours and tastes (Astray et al., 2009). Their ability to decrease bitterness is due to their hydrophobic cavity and hydrophilic exterior shell (Szejtli and Szente, 2005), where bitter eliciting compounds interact with the interior of cyclodextrin, forming an inclusion complex that rapidly dissociates upon contact with gastric juice (Szejtli, 1988). As a result, bitterness is decreased, as bitter eliciting compounds are complexed within the cyclodextrin molecule, and thus are unable to bind to TRCs.

β-cyclodextrins (β-CD) have the lowest water solubility of all three types of CDs, most likely due to their rigid intramolecular hydrogen bond formation (Astray et al., 2009). However, their lower production cost and increased efficiency in bitterness reduction compared to α- and γ-CDs, make them the most commonly used form in the industry (Szejtli, 1988). This, along with their GRAS recognition (Szente and Szejtli, 2004), makes them an especially attractive choice for bitterness inhibition with functional foods and nutraceuticals.

β-Cyclodextrins have been successfully used to decrease bitterness when applied to a variety of food products including citrus juices. For example, the use of 0.3 and 0.5% β-cyclodextrin substantially decrease the bitterness intensity of citrus juice (Konno et al., 1982). Similiar findings in bitterness reduction have resulted when β-cyclodextrin was polymerized and used to remove limonin and naringin from aqueous solutions, as well as from citrus juices via continuous flow processes (1 g of β-cyclodextrin polymer reduced the limonin content in 55 mL of filtered orange juice from 8 to 4 ppm in 15 min, with similar efficiency for removal in grapefruit juice) (Shaw and Wilson, 1983). Also, the addition of 0.5% β-cyclodextrin to aqueous solutions containing naringin and limonin decreased their bitterness intensity by approximately half (Konno et al., 1982).

Cyclodextrins may be valuable in decreasing the bitter taste elicited by various wine derived polyphenolics such as *trans*-resveratrol and (+)-catechin - health-promoting compounds (Renaud and de Lorgeril, 1992; Frankel and Kanner, 1993) that are attractive candidates for fortifying foods and beverages. However, consideration should be given in product formulation to the ability of β-cyclodextrin to elicit sweetness at higher concentrations (.039% and .11% detection and recognition thresholds, respectively) (Toda et al., 1981). In addition, cyclodextrins

can alter the sensory profile of a product through flavour encapsulation (Astray et al., 2009), potentially limiting their use at higher concentrations. Further, if used in functional food formulations with other bitter suppressing ingredients, cyclodextrins may interfere with the blocking mechanisms of these agents.

Riboflavin binding protein

Riboflavin binding protein (RBP), found in chicken eggs, transports and stores riboflavin (vitamin B_2), providing necessary nutrients during the growth and development of the chick embryo (Croguennec et al., 2007) (Figure 2.5).

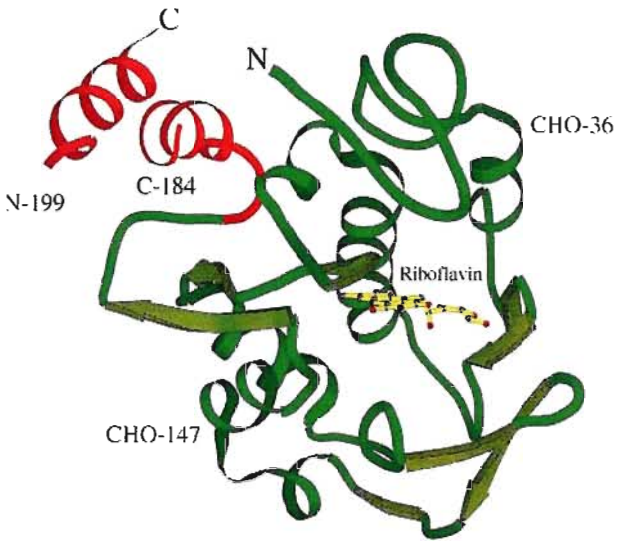


Figure 2.5. Ribbon structure of riboflavin binding protein (Monaco, 1997).

RBP is a potent bitter inhibitor, significantly decreasing the bitter taste intensity of numerous compounds, including naringin, denatonium, caffeine, theobromine, glycyl-L-phenylalanine, and QHCl (Maehashi et al., 2008). For instance, the addition of 0.2mM RBP

decreased the bitterness of 0.125 mM solution of QHCl by nearly 100% (Maehashi et al., 2008).

RBP has been shown to bind to QHCl via hydrophobic interactions, and thus, decrease its bitterness, although it suppresses the perception of other bitter compounds via competition for sites on bitter taste receptors (Maehashi et al., 2008). Thus, depending upon the bitterant, RBP has more than one mode of action accounting for its bitterness reducing capacity.

In addition to its ability to suppress bitterness, RBP inhibits sweetness elicited by proteins such as thaumatin, monellin and lysozyme (Maehashi et al., 2007). However, the perception of other sweeteners, some of them commonly used in the food industry, is not inhibited: sucrose, glycine, D-phenylalanine, saccharin, cyclamate, aspartame, and stevioside.

These findings suggest that RBP selectively inhibits sweet eliciting compounds, but has the capacity to suppress the taste of a wide range of bitterants. This combination would be desirable for use with functional foods, where the sweetness of commonly used sweet eliciting compounds would remain, further contributing to the suppression of bitterness due to central cognitive effect processes (Kroeze and Bartoshuk, 1985; Keast and Breslin, 2002a).

Flavanones

Some flavanones extracted from the shrub Herba Santa (*Eriodictyon californicum*) (Geissman, 1940) can decrease the bitterness of caffeine without eliciting strong side tastes or flavours (Ley et al., 2005). In particular, 100 ppm eriodictyol and 100 ppm homoeriodictyol sodium salt lower the bitterness of 500 ppm caffeine by approximately 45% and 40%, respectively (Ley et al., 2005).

On average, homoeriodictyol sodium salt decreases the bitterness of a wide range of structurally different bitterants (guaifenesin, paracetamol, quinine, denatonium benzoate, salicin and amarogentin) by 35% (Ley et al., 2005). In addition to its bitter blocking capacity, homoeriodictyol sodium salt does not affect the perception of saltiness or sweetness (Ley et al., 2005), which may be particularly valuable in sweet or salty tasting food and beverage formulations (Figure 2.6).

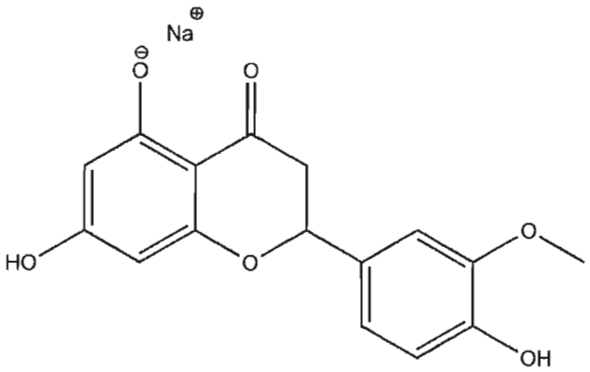
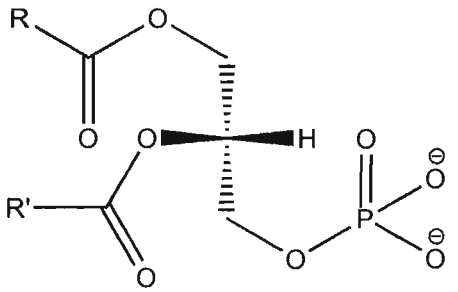


Figure 2.6. Chemical structure of homoeridictyol sodium salt.

The mode of action by which homeoriodictyol sodium salt and other Herba Santa flavanones decrease bitterness is currently unknown. However, since other tastes are not affected, it is postulated that these compounds target specific sites on TAS2Rs rather than cellular transduction pathways that are common to bitter, sweet, and umami tastes. Overall, bitter blocking flavanones from natural herbal sources could be very attractive in functional food formulations, although further studies are needed on their efficacy with bitter functional ingredients.

Phosphatidic acid - ß-lactoglobulin

Lipoproteins composed of phosphatidic acid (PA, a phospholipid) and ß-lactoglobulin (LG, a protein) (PA-LG) can decrease the bitterness of a range of bitter eliciting pharmaceuticals, yet do not inhibit the saltiness of sodium chloride or the sweetness of sucrose (Katsuragi & Kurihara, 1993; Katsuragi et al., 1995) (Figure 2.7). PA alone can also decrease the bitterness of QHCl by approximately 81% (Nakamura et al., 2002). The suppression of QHCl from PA is hypothesized to be due to both blocking of *TAS2Rs* [most likely due to PAs high affinity for hydrophobic regions on TRCs (Katsuragi et al., 1996)], and adsorption to the compound itself (Nakamura et al., 2002).



A

B

Figure 2.7. Structures of phosphatidic acid (A) and ß-lactoglobulin (B).

A similar mechanism for bitter suppression is possible for lipoproteins. An increase in the

concentration of PA-LG (from 0.3% to 1%) applied as a pre-treatment to the frog glossopharyngeal nerve increases bitter suppression when 0.1mM QHCl and 1 mM papaverine hydrochloride solutions are administered. This provides evidence that elevated bitter suppression may be due to an increased binding of PA-LG to target sites on TRCs (Katsuragi et al., 1996).

Although PAs bitter suppressing capabilities are apparent, it is relatively insoluble in water compared to lipoproteins such as PA-LG (Katsuragi et al., 1995), which may limit its suitability for aqueous functional food products. Since PA is derived from soybeans, and LG from milk and eggs, PA-LG may have a wider acceptance as a bitter-blocker in functional foods (Katsuragi et al., 1995). However, due to potential allergens associated with PA-LG (Wal, 1998; Sicherer et al., 2000; Savage et al., 2007), its usage may be limited for some food and beverage formulations.

Neodiosmin

Neodiosmin, found in citrus fruit, is a glycosylated flavone (Del Rio et al., 1992). It is derived from the bitter flavanone, neohesperidin, and at low concentrations, is tasteless and odourless (Guadagni et al., 1976) (Figure 2.8).

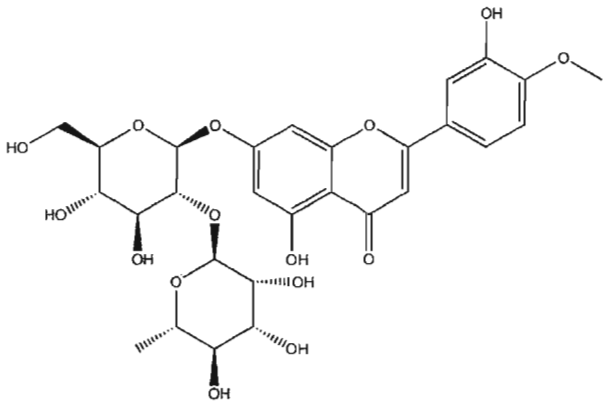


Figure 2.8. Chemical structure of neodiosmin.

The threshold of aqueous solutions of caffeine, quinine sulfate, and naringin can be increased by approximately 80%, 137%, and 225%, respectively, with the addition of 10 ppm neodiosmin (Guadagni et al., 1976, 1979). 10 ppm neodiosmin also increases the threshold of limonin by 53% in water and 300% in orange juice (Guadagni et al., 1976).

As neodiosmin is tasteless up to the maximum concentration tested of 40 ppm (Guidagni et al.,1976), and at lower concentrations effectively suppresses the bitterness of various compounds, it may be an especially attractive addition to functional food formulations.

Zinc salts

Low molecular weight compounds, such as zinc lactate and zinc sulfate ($ZnSO_4$), significantly decrease the bitterness elicited by a range of bitterants including caffeine, QHCl, Tetralone, and denatonium benzoate (Keast and Breslin, 2005; Keast, 2008). The bitter suppressing effect of $ZnSO_4$ (25 mM) on range of QHCl concentrations (0.04 -0.4 mM) is

particularly impressive (70%) (Keast, 2003). It is postulated that through interaction with specific amino acids (serine, threonine) on the extracellular portion of some bitter taste receptors, $ZnSO_4$ may alter the integrity of *TAS2R* receptors, preventing them from functioning normally (Keast, 2003).

$ZnSO_4$ also significantly decreases sweet taste intensity of a variety of compounds frequently used in the food industry, including sucrose, glucose, fructose, aspartame, saccharin, and sucralose (Keast, 2003; Keast et al., 2004; Keast and Breslin, 2005). The inhibition of both bitter and sweet taste may not be desirable in functional foods that impart sweetness, as sweetness can mask the perception of bitterness due central cognitive processing (Keast and Breslin, 2002a). However, $ZnSO_4$ may be useful in savory formulations where an inhibition of sweet taste is inconsequential. In addition, zinc fortification of foods is an attractive and active strategy for decreasing zinc deficiency in the population (Salgueiro et al., 2002), and may confer additional value to the use of $ZnSO_4$ in functional foods.

Magnesium sulfate

Magnesium sulfate ($MgSO_4$) has been shown to decrease bitterness of QHCl by approximately 52% without affecting the perception of other basic taste qualities (Keast, 2003), providing support for its use to decrease the taste intensity of a wider range of bitter functional formulations compared to that of $ZnSO_4$. However, it is not clear that $MgSO_4$ will suppress the bitterness of health-promoting functional ingredients such as (+)-catechin, as the bitter inhibiting capacity of $MgSO_4$ is compound dependent (Keast and Breslin, 2005).

Although $MgSO_4$ itself has been used as a bitter stimulus in bitter taste research (Keast and Breslin, 2002b), and can elicit other taste qualities such as sour and salty (Delwiche et al., 2001), the concentrations used in these studies (0.78 M and 0.3 M, respectively) are higher than used by Keast (2003) in establishing its bitter suppressing activity (0.025 M). One study found that $MgSO_4$ elicits basic tastes and other non-taste qualities (metallic) at 0.025 M, but the intensity of these oral sensations are minimal (Lawless et al., 2003). Therefore, $MgSO_4$ may be of value in decreasing the bitterness of functional ingredients due to its significant and specific inhibition of bitter taste, and the minimal elicitation of other oral sensations if used at concentrations less than 0.025M.

Fatty acids

While the underlying molecular mode of action that lipids may have on taste perception is unclear, there are various studies that demonstrate the impact that fats and oils have on overall taste sensation, including bitterness. In simple solutions, lipids may mask or suppress bitterness, however, the purported suppressing ability of lipids in real food products is equivocal.

In paired comparison tests, caffeine and quinine are more easily detected in water compared to peanut oil solutions, providing evidence for the bitter masking ability of lipids (Mackey, 1958).

The bitterness of caffeine solutions is reduced and their taste threshold is increased by 160% when 1% linoleic acid is added (Mattes, 2007), suggesting that this particular fatty acid may mask the bitter taste of caffeine.

When assessing bitter intensity using a 10-point line scale, high (0.085 M) and low (0.0085 M) concentrations of quinine sulfate and oil are rated lower compared to quinine and water (Metcalf and Vickers, 2002). Specifically, the bitterness of low quinine in 9% oil is decreased by 21%, while high quinine is reduced by 10%. In 17% oil, bitterness of low quinine is unchanged, but high quinine is decreased by 15% (Metcalf and Vickers, 2002).

Time-intensity methods demonstrate that prior rises with sunflower and coconut oils significantly reduce the maximum intensity, total duration, and total area under the curve of bitterness elicited by high (9.0×10^{-3} M) and low (4.0×10^{-5} M) concentrations of quinine sulfate presented in gelatin form (maximum intensity lowered by approximately 22 and 40% for high and low concentrations, respectively) (Lynch et al., 1993). Similarly, the maximum bitterness of QHCl was decreased by 25%, and other time intensity parameters were reduced following ingestion of sunflower oil (Valentová and Pokorný, 1998).

In one study, tuna, soybean, and high oleic acid corn oils were emulsified with compounds eliciting basic tastes (Koriyama et al., 2002). While the taste intensity of some compounds were increased by certain oils, (e.g., monosodium glutamate in tuna oil), oils generally decreased the maximum taste intensity of sour and bitter eliciting compounds. Tuna oil, which is rich in the omega-3 fatty acid decosahexaenoic acid (DHA), was most effective at suppressing the maximum taste intensity of quinine sulfate. Of interest, the 3 oils did not differ in their ability to suppress leucine, a bitter tasting amino acid commonly found in tuna extract (Koriyama et al., 2002). Therefore, fatty acids have the ability to significantly modify the perception of various tastants, with the size of the effect dependent on the chemical nature of the fatty acid.

It has been postulated that lipids may influence taste perception by acting as a physical barrier, or 'mouthcoat', between taste eliciting compounds and TRCs, and thus, decreasing

overall taste intensity (Lynch et al., 1993). Alternatively, lipids may increase the concentration of non-polar taste eliciting compounds in the water phase, and thus, increase the intensity of perceived taste (Yamamoto and Nakabayashi, 1999). While the barrier hypothesis would explain the masking effect of lipids on bitterness, this explanation would generally not support an increase in bitterness perception, as many bitter eliciting compounds are hydrophobic rather than hydrophilic in nature (Funasaki et al., 1999). In addition, it has also been suggested that fatty acids influence taste perception via modulation of TRCs, as extracellular application of some fatty acids significantly inhibits outward delayed rectifying K^+ currents in TRCs, leading to a prolonged states of depolarization and hence, and excitable state in the cell (Gilbertson et al., 1997).

Overall, fatty acids may provide an interesting option for bitterness masking in functional foods. Being non-polar, they may be well suited to carry additional hydrophobic functional ingredients in functional beverage formulations. Also, many fatty acids, including omega-3s, are highly nutritive, and could provide additional functionality to products. For instance, omega-3 fatty acids are linked to prevention of many inflammatory related diseases, including cardiovascular disease, diabetes, and stroke. DHA in particular is believed to be crucial to proper brain and retina development (Simopoulos, 1991, 1997; Kris-Etherton et al., 2003). However, fatty acids are particularly susceptible to oxidation, and thus, can impart negative sensory attributes to some food formulations (Kolanowski et al., 1999). Thus, in most instances, omega-3 fortification would require incorporation of antioxidants or other strategies to maintain chemical stability and functionality.

CONCLUSION AND FUTURE DIRECTIONS

Foods and beverages that elicit excessive bitterness are generally unacceptable to consumers. Some functional ingredients, including some polyphenolics, are bitter, and thus their use in functional foods may result in undesirable levels of bitterness, particularly in products that already elicit a bitter taste. Various approaches can be employed to reduce or mask the bitterness elicited by these products, such as the use of basic taste eliciting compounds, odourants, textures, and bitter blocking compounds.

Sweet, salty or umami-eliciting compounds can be incorporated into products to help decrease bitterness, with varying degrees of success. One disadvantage, however, of adding some sweeteners, salts and monosodium glutamate is their negative association with health, which may therefore reduce the perceived value of the 'health-promoting' functional foods to which they have been added. Texture-modifying ingredients, such as vegetable gums, can also be effective at reducing bitterness. Odourants may also be used, contributing to the overall flavour while helping to mask bitterness perception. To date, however, there is little literature informing which odourants may be effective with bitterness. Bitter blockers – with the potential of larger effect sizes and greater specificity - represent the most promising approach to reducing the bitterness of functional foods in the future.

Bitter blockers represent a diverse range of chemical classes that also vary in their mode of action. Some out-compete bitterants at *TAS2R* binding sites, while others prevent their interaction with *TAS2R*s by complexing with or encapsulating them. Their relative efficacy varies with, amongst other considerations, the bitterant, the food/beverage matrix and capacity to elicit their own taste or flavour. Thus, careful matching of bitter blocker to the functional food is needed in product formulation, and requires more research. The existence of different taste

phenotypes in a target population may have implications for the optimal bitterness suppression strategy, and also warrants further examination.

Overall, various applications are currently used in the food industry to decrease bitter taste sensation. While traditional methods remain valuable, recent advances in the formulation and testing of bitter blocking compounds promise new and potentially more effective methods to inhibit bitterness. They may have particular application with new, bitter-eliciting functional ingredients, including polyphenolics, which are being introduced into functional foods and beverages. Future strategies for bitterness modification could involve the use of bitter blockers alone, or in conjunction with more traditional approaches, and ultimately provide more effective options for the dynamic functional food sector.

Chapter 3 - SENSORY AND CHEMICAL CHARACTERISTICS OF *TRANS*-RESVERATROL FORTIFIED WINE

Nicole J. Gaudette and Gary J. Pickering

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INTRODUCTION

As the fastest growing segment in the food industry (Verbeke, 2005), functional foods and beverages represent an attractive alternative to conventional food products for health conscious individuals. Through the incorporation of fortifying agents, including pre- and pro-biotics (Luckow and Delahunty, 2004) and antioxidant-eliciting ingredients (Lavelli et al., 2010), ordinary foods are being developed into 'superfoods' (Lunn, 2006), providing increased physiological benefits and/or reducing the risk of chronic disease beyond basic nutritional functions (Health Canada, 2002). Functional beverages and plant-based fortified products are especially attractive to consumers (Boue et al., 2009), and represent novel opportunities for beverage producers.

One plant-derived compound receiving considerable attention in the biomedical literature is *trans*-resveratrol (3,4,5' trihydroxy-*trans*-stilbene; *t*R); a stilbene and polyphenol that can be found in peanuts (Tokusoglu et al., 2005), strawberries (Wang et al., 2007), blueberries and bilberries (Lyons et al., 2003), and grapes (Siemann and Creasy, 1992). A number of positive health effects have been associated with *t*R, including protection against diabetes (Palsamy and Subramanian, 2008), colon and breast cancers (Schneider et al., 2000; Nakagawa et al., 2001), and cardiovascular disease via inhibition of LDL oxidation, increased endothelial nitric oxide production, and inhibition of platelet aggregation (Frankel and Kanner, 1993; Wallerath et al., 2002; Wang et al., 2002). *t*R also significantly decreases the negative effects of a high-caloric diet (Baur et al., 2006), and provides neuroprotection via upregulation of endogenous antioxidant expression and activity (Robb and Stuart, 2010).

*t*R exists in a variety of foods, perhaps most notably in wine. Originating in the skin of grapes, *t*R is a phytoalexin; a compound produced by the vine that acts similarly to an antibiotic in response to attack from stressors such as the grape fungus *Botrytis cinerea* (Jeandet et al., 1995b). *t*R is extracted into wine during vinification, where it is found in four forms - *trans*- or *cis*-aglycone and *trans*- or *cis*-glycoside, mediated by enzymatic isomerisation and hydrolytic reactions (Mattivi et al., 1995).

On average, red wine contains 7 mg/L of total resveratrol, rosé wine 2mg/L, and white wine 0.5mg/L (Waterhouse, 2002), although there are significant differences between grape varieties (Pour Nikfardjam et al., 2006, Lee and Rennaker, 2007), primarily attributed to the physiological characteristics of the variety itself, growing conditions, fungal pressure, and climatic variables (Goldberg et al., 1995 a,b).

While white wines are not a significant source of *t*R, some vinification methods, including increased skin maceration time, have been employed to increase their polyphenolic content and antioxidant capacity (Fuhrman et al., 2001). Various vinification practices can also elevate the final concentration of *t*R in red wine, specifically; increased maceration time (Gambuti et al., 2004), pomace pressing (Gambuti et al., 2004), malolactic fermentation (Vrhovsek et al., 1997), pre-fermentation cold soaks (Clare et al., 2004), and thermovinification (Clare et al., 2004). Post-harvest, controlled, ultraviolet-irradiation can increase stilbene synthesis in grapes, producing fruit with ten-fold higher *t*R concentrations compared to control (Cantos et al., 2001), and wine with up to two times higher *t*R concentrations (Cantos et al., 2003). Short, anoxic treatments of 6 and 15 hours to grapes using dry nitrogen, increase the endogenous levels of *t*R in grapes and their subsequent wine without significantly affecting other wine quality measures (Jimenez et al., 2007). Purified ß-glucosidase from *Aspergillus niger* can

increase levels of *tR* in wine by converting *trans*-piceid to *tR* aglycon (Todaro et al., 2008), with similar results when transgenic yeasts expressing a glycosyl-hydrolase are used during fermentation (Gonzalez-Candelas et al., 2000). Reduction in wine *t*R concentration may also occur during winemaking. When the maximum recommended levels of bentonite, egg white, gelatin + kieselsol or polyvinylpolypyrrolidone (PVPP) are used for fining, resveratrol levels decrease significantly compared to control, with the size of the effect dependent on the type and concentration of fining agent and grape variety (Threlfall et al., 1999 a,b). *t*R concentration can also decrease after filtering (Soleas et al., 1995).

Many of these cultural approaches that result in higher *t*R concentration in wine also increase levels of other grape-derived phenolic species, particularly (+)-catechin and (-)-epicatechin. Concurrent with this is the potential for excessive bitterness and astringency elicited by these compounds (Peleg et al., 1999) and lower consumer acceptance of the wine (Lesschaeve and Noble, 2005). Thus the interest in more selective approaches, including engineering higher *t*R in grapes through genetic and traditional breeding methods, and direct fortification of *t*R into wine. The latter approach is already finding commercial application (Norrie, 2006). The stability, however, of *t*R in wine is not well known, although in general phenolics undergo significant changes during wine aging, including self-association, polymerization, co-pigmentation, and precipitation reactions (Waterhouse, 2002). Soleas et al. (1995) reported a decrease in *t*R concentration of approximately 40% in a juice fermented over a 63 day period. However, we are not aware of any literature that examines the chemical and sensory characteristics of wines with elevated *t*R. These considerations form the basis of this study.

MATERIAL AND METHODS

Wine preparation

Base wines were prepared from commercial kits of juice concentrates (Cabernet Sauvignon, "California Connoisseur"; Riesling, "European Select"; Vineco International Products Ltd., St. Catharines, Ontario). The typical processing regime employed in the manufacture of these concentrates is as follows: Grapes are pressed for 2-4 hours in a Bucher bladder press, and enzymes are added for colour and flavour extraction. The juice is held at 28 °C for 4-6 days for settling under a nitrogen environment. Racked juice of less than 0.01% solids is pumped to holding tanks for concentration, while juice with higher solid content is filtered using diatomaceous earth. The juice is then evaporated using a FMC Food Tech® 4 stage evaporator. Pasteurization is the first stage of this process, and the evaporator is a continuous feed steam-driven closed system employing an average temperature of 120 °C. The concentrate is cooled through a plate and frame exchanger upon exit of system, and stored in stainless steel tanks and temperature controlled rooms.

After receiving the juice, we chaptalized it using super fine sucrose (Lantic Sugar Ltd., Montreal, Quebec) to achieve a desired alcohol content of approximately 14%. This target ethanol concentration was established after bench-testing in order to ensure full solubility of the planned 200 mg/L *t*R addition (data not shown). Juice concentrates were rehydrated and inoculated using yeast strain EC1118 according to the manufacturer's instructions (Lallemand Inc., Rexdale, Ontario). Stainless steel fermentation vessels were used for fermentation, with chamber temperatures at 20°C and 18°C for Cabernet Sauvignon and Riesling wines, respectively. The Cabernet Sauvignon wine was treated by hanging cheesecloth containing 1 g/L

Hungarian oak medium plus toast chips (StaVin, Inc., CA, USA) from days 2 - 8 of fermentation to approximate the flavour characteristics of most commercial Cabernet Sauvignon wine.

Following fermentation (12 days Cabernet Sauvignon, 14 days Riesling), wines were racked into glass carboys, SO_2 levels were adjusted to obtain 25-30 mg/L free SO_2, and carboys were transferred to a -2°C holding chamber for 21 days to cold stabilize. Wines were then filtered into clean, glass carboys using a 1μm filter pad followed by a 0.45 μm membrane filter.

After filtration, 0 mg/L (control), 20 mg/L (treatment 1), or 200 mg/L (treatment 2) of *t*R was added to the carboys. A stock solution of 99% pure food grade *t*R (Chromadex, California, USA) was first solubilized in 95% food grade ethanol (Liquor Control Board of Ontario, St. Catharines, Ontario) at its limit of solubility (50 mg *tR*/mL ethanol). Due to the degradation of *t*R by UV light (Trela and Waterhouse, 1996), a foil wrapped Erlenmeyer flask was used to solubulize *t*R into ethanol. Required amounts of the stock solution were then immediately added to filtered wines. Fortification occurred after filtering to avoid possible loss of *t*R from membrane filtration (Soleas et al., 1995). SO_2 levels were adjusted again to obtain 25-30 mg/L free SO_2, and the wine bottled into green, 750 mL Bordeaux style bottles, sparged using nitrogen gas, and closed using 38 mm length, UFB, Sterisun® corks (Pickering, Ontario). Wines were then stored in a dark wine cellar controlled for temperature (mean temp 12°C) and humidity until required for analysis.

Chemical analyses

Basic wine chemical analyses, including pH, titratable acidity (TA), free and total SO_2, and ethanol were performed on wine sampled directly from cellared bottles. Antioxidant

capacity, resveratrol, and spectrophotometric measurements were performed on frozen samples that were gently thawed using a warm water bath. Frozen samples were prepared at each time point from cellared bottles that were used for the above analyses. Wine was transferred into 125 mL Nalgene® HDPE bottles (Sigma-Aldrich, Oakville, ON) under nitrogen gas, and sample bottles were tightly closed and covered with laboratory film (Parafilm "M", Pechiney Plastic Packaging, IL, USA) and immediately frozen (-18°C) for later analysis.

Basic wine chemistry and antioxidant capacity

Standard wine chemical analyses were conducted on 2 bottles from each treatment and in duplicate at bottling and at 6, 18, 31, 44, and 58 weeks post bottling. pH was determined using a Fisher Scientific AB15/15+ Accumet Basic pH meter. TA, free and total SO_2, ethanol (ebulliometry), and wine phenolics were measured after Iland et al. (2003). Antioxidant capacity was determined using the TEAC method, which spectrophotometrically measures the decolourization of a blue-green 2,2'-azino-bis(3-ethylbenzthiazoline-6-sulphonic acid (ABTS) radical via the scavenging ability of an antioxidant compared to the vitamin E derivative, Trolox™ (Sigma-Aldrich, USA) (Pour Nikfardjam, 2002).

Resveratrol

Reversed-phase high performance liquid chromatography (HPLC) was used to quantify *trans* and *cis*-resveratrol (Ibern-Gomez et al., 2002). A Hewlett-Packard (HP) series 1100 gradient UV/Vis liquid chromatograph with diode-array detector and corresponding HP

ChemStation for LC software (Rev.A.07.01 [682]) was used for detection and analysis. An Agilent (Agilent Technologies, USA) 50 x 4.6 mm, 3.5 μm Zorbax SB-C18 column at 30°C was used to separate *cis*- and *trans*-resveratrol. Mobile phases A and B consisted of a 0.2% trifluroacetic acid solution [TFA, 99%+ spectrophotometric grade (Sigma-Aldrich, USA)] in pure water (Millipore RiOs 16 Reverse Osmosis System, MA, USA) and 99% pure HPLC grade acetonitrile (Celadon, USA), respectively. Standards and wine samples were directly injected at a volume of 10 μL, a flow rate of 1 mL/min, and a run time of 26 min with a 10 min post run. The following gradient was used: 0-15 min, from 5% to 35% B; 15-16 min, from 35% to 100% B; 16-25 min, 100% B; 25-26 min, from 100 to 5% B. Chromatographic separation of *trans* and *cis*-resveratrol was determined using 306 and 285 nm, respectively (Montsko et al., 2008).

Samples were immediately transferred from Nalgene® bottles to 2 mL amber, snap top chromatography vials (Agilent Technologies, USA). Vials were kept refrigerated at 4°C until sample preparation was completed (held no longer than 1 hour prior to HPLC injection). 8-point standard curves for both *trans* ($r = .999$) and *cis*-resveratrol ($r = .968$) were created (1.56 mg/L, 3.125 mg/L, 6.25 mg/L, 12.5 mg/L, 25 mg/L, 50 mg/L, 100 mg/L, and 200 mg/L) using 99% pure HPLC grade *t*R (Sigma-Aldrich, USA) dissolved in acidified ($pH<3.0$) 99% HPLC grade methanol (Fisher Scientific, USA). As *cis*-resveratrol is not commercially available, it was synthesized from *t*R using the isomerization procedure of Lopez-Hernandez et al. (2007). In a dark room and at a distance of 60 cm, a long wave UV light (115 Volts, 60Hz, 0.16 Amps) (Mineralight Lamp, UVG-54, Ultra-Violet Products, Inc., CA, USA) was shone for 10 min onto UV-Vis cuvettes containing *t*R standards. Samples were then transferred immediately to amber chromatography vials, sealed, and kept under refrigeration until analysis (same day). Successful synthesis of the *cis*-aglycone was verified in all samples by the detection of 2 peaks (*trans* and

cis) at 285 nm (Ibern-Gomez et al., 2002). Two separate 20 mg/L *t*R standards were prepared fresh and included at the beginning and at the end of each HPLC run in order to monitor retention time drift.

Sensory analyses

Participants for all sensory evaluation sessions were faculty, staff, or students of Brock University's Cool Climate Oenology and Viticulture Institute (CCOVI). All panelists volunteered their time and signed consent forms that had been cleared by Brock University's Research Ethics Board. Sessions were held in CCOVI's wine sensory evaluation lab, equipped with a computerized program for data collection (Compusense c5v4, Guelph, Ontario, Canada).

Difference testing – flavour and aroma evaluation

Triangle tests were performed using untrained panelists at 6 (n = 27) and 32 weeks (n = 42) post bottling (Lawless and Heymann, 1998; Mielgaard et al., 1999) to determine whether flavour or aroma differences existed in tR enriched wines compared to control. Separate sessions were conducted for Cabernet Sauvignon and Riesling wines (2 sessions for each wine style). At least a 30-minute break between sessions was enforced, with panelists participating in no more than 2 sessions per day. Each session consisted of 2 flights of 3 wines in a balanced, randomized design, for a total of 6 wines per session. The first flight of samples compared 20 mg/L wines compared to control, and the second flight compared 200 mg/L wines to control. 30 mL wine samples were presented in black tasting glasses (Spiegelau, USA) labeled with randomized 3-digit numbers. To decrease possible carryover effects, unsalted crackers and

filtered water were required as palate cleansers during the forced 3-minute break between flights. In addition, a 5 g/L pectin rinse, followed by a water rinse, was used during the forced break for Cabernet Sauvignon sessions to reduce possible interference from astringency carryover (Colonna et al., 2004). Panelists were instructed to refrain from consuming any strongly flavoured foods and beverages for at least 30 minutes prior to participation, and were required to expectorate all wine samples during data collection.

Difference testing - visual evaluation

Triangle tests to determine possible visual differences between wine treatments were also conducted at 6 (n = 13) and 32 weeks (n = 18 and 23 for Cabernet Sauvignon and Riesling, respectively) post bottling. Since visual sensory evaluations are less fatiguing compared to taste and aroma evaluations, all testing was conducted during a single session. Riesling and Cabernet Sauvignon were evaluated separately and in duplicate in 2 flights with 3 samples per flight. Wines were assessed using standard colourless ISO tasting glasses.

Descriptive analysis

After the results were obtained from the difference testing, descriptive analysis (Lawless and Heymann, 1998) was conducted on Riesling wines at 16 weeks (n = 6) and 40 weeks (n = 10) post bottling (no significant difference were found between Cabernet Sauvignon wines). Panelists were recruited based upon their performance on previous triangle tests (ability to correctly choose the 'different' sample in at least 2 out of 4 flights tested). The first 4 sessions

involved panel training, consisting of familiarization with the wines, formulating a lexicon of basic taste, flavour, mouthfeel and odour descriptors, and practicing the use of line scales. Data

Table 3.1. Riesling aroma and flavour descriptors with corresponding reference standard compositions.

Descriptor	**Reference composition***
citrus	5 mL fresh lemon juice + 50 mL wine
apple	10 g chopped Granny Smith apple + 30 mL wine
vegetal	1 mL stock solution + 40 mL wine (stock = 1.1 g fresh wheat grass blended with 100 mL water)
stonefruit	5 mL peach nectar (Yoga®, Italy) + 2 mL apricot nectar (Yoga®, Italy) + 50 mL wine
floral	1 bunch fresh red freesia
candied banana	20 mL stock + 10 mL wine (stock = 1 drop isoamylacetate [Sigma-Aldrich, USA] + 150 mL wine)
ripe banana	1 peeled, ripe banana
honey**	7.5 g honey (Billy Bee Honey Products, Toronto, Canada) + 50 mL wine
acidity	1.5 g/L citric acid in aqueous solution (Sigma-Aldrich, USA)
bitterness	0.01 g/L quinine sulfate in aqueous solution (Sigma-Aldrich, USA)
salty	2.5 g/L pure coarse salt in aqueous solution (Windsor, Quebec, Canada)
heat	14 mL 95% pure food grade ethanol into 100 mL water (Liquor Control Board of Ontario, St. Catharines, Ontario)
astringent	0.5 g/L aluminum sulphate in aqueous solution (Sigma-Aldrich, USA)

* Reference standards were prepared using neutral Pinot Grigio base wine (CCOVI Pilot Winery) unless otherwise indicated. Standards were freshly prepared 12 hours before each session for full aroma development, stored at 4°C, and moved to room temperature 1 hour prior to session.

** Prepared 30 min before each session.

collection took place over 3 separate sessions. At least a 30-minute break between sessions was enforced, with panelists participating in no more than 2 sessions per day. The same tasting, rinsing, and sample preparation protocols used for difference testing were implemented. Each session included 1 flight of 3 wines (control, 20 mg/L, 200 mg/L Riesling) in a balanced, randomized design. Panelists evaluated each wine sample first for aroma (orthonasal), then for flavour attributes. Attributes were rated for intensity on a 15 pt line scale, with "absent" and "high" representing the bottom and top anchors, respectively. Reference standards for each descriptor were developed during panel training, and were assessed at the start of each session (Table 3.1). Each standard was indicated on the respective line scales at the appropriate intensity agreed on by the panel.

Statistical analyses

Chemical

Statistical analysis was performed using XLSTAT version 2008.6.01 for Apple Macintosh (Addinsoft, USA). Two-way ANOVAs were conducted for each time point and treatment. Bottle and interaction were included as the independent variables in the initial analysis, which was repeated with bottle removed if it was not significant ($p(F)>0.05$). Fisher's LSD mean separation tests were used for post-hoc analyses ($\alpha=0.05$) following a significant ANOVA ($p(F)<0.05$).

Sensory

Analysis of the data from the difference tests was conducted using tables of the critical number of correct responses in a triangle test needed for significance (Meilgaard et al., 1999).

Analysis of descriptive analysis data was performed using SPSS version 16.0 for Apple Macintosh (SPSS Inc., Chicago, USA). A linear mixed model ANOVA with repeated measures was used to examine whether descriptor intensity ratings differed between wine treatments. In the model, judge was treated as a random effect, treatment as a fixed effect, and rep as a repeated effect. Tukey's HSD mean separation tests were used for post-hoc analyses (α=0.05) following a significant ANOVA ($p<0.05$).

RESULTS

Chemical analyses

Basic wine chemistry and antioxidant capacity

Basic wine chemistry analyses, including TA, SO_2, and pH were performed at each time point on cellared wine. Spectrophotometric, antioxidant capacity, and HPLC analyses were performed on thawed, frozen samples. We hypothesized that pH and TA would not be affected by *t*R fortification, and that free SO_2 levels would be higher than control due to the additional antioxidant protection provided by *t*R. The results for TA, SO_2, and pH for Cabernet Sauvignon and Riesling wines are represented in Table 3.2.

*t*R fortification significantly affected TA, as values were higher for 20 mg/L and 200 mg/L Riesling compared to control at some time points (18, 31, and 44 weeks). Small

differences in TA were also evident for Cabernet Sauvignon across treatment and time. Free SO_2 concentrations decreased over time for all treatments, which is expected due to the capacity of SO_2 to reduce oxidative products and react with the oxygen that slowly migrates into wine over time (Margilit, 2007). Generally, free SO_2 levels were not significantly affected by level of *t*R fortification, although small differences were observed for Riesling at 58 weeks, and Cabernet Sauvignon at 31 and 58 weeks. *t*R fortification did not affect pH.

Antioxidant capacity for all wines, determined using the TEAC assay, is given in Figure 3.1. We hypothesized that *t*R fortification would increase the antioxidant activity of both Cabernet Sauvignon and Riesling wines. For most time points, TEAC values were higher for 20 mg/L and 200 mg/L Cabernet Sauvignon wines compared to control. At all time points, 200 mg/L Riesling wine had significantly higher TEAC values than control. It is possible that some of the differences between the white and red wines in their antioxidant capacity may be attributable to synergistic interactions with other phenolic compounds.

Spectrophotometric estimates - wine colour and phenolic composition

Wine colour and phenolic composition of Cabernet Sauvignon and Riesling wines are represented in Table 3.2 and 3.3. Wine colour hue and density for Cabernet Sauvignon were significantly different from control for both 20 mg/L and 200 mg/L at all time points, with the exception of 18 weeks for wine colour hue, where differences were observed for 200 mg/L only. Wine colour density was higher at all time points with increasing level of *t*R fortification, while wine colour hue was lower, suggesting *t*R is associated with a higher ratio of red to yellow/brown coloured pigments. Total red pigments significantly decreased for each treatment over time, as pigmentation shifted from red/blue to yellow/brown.

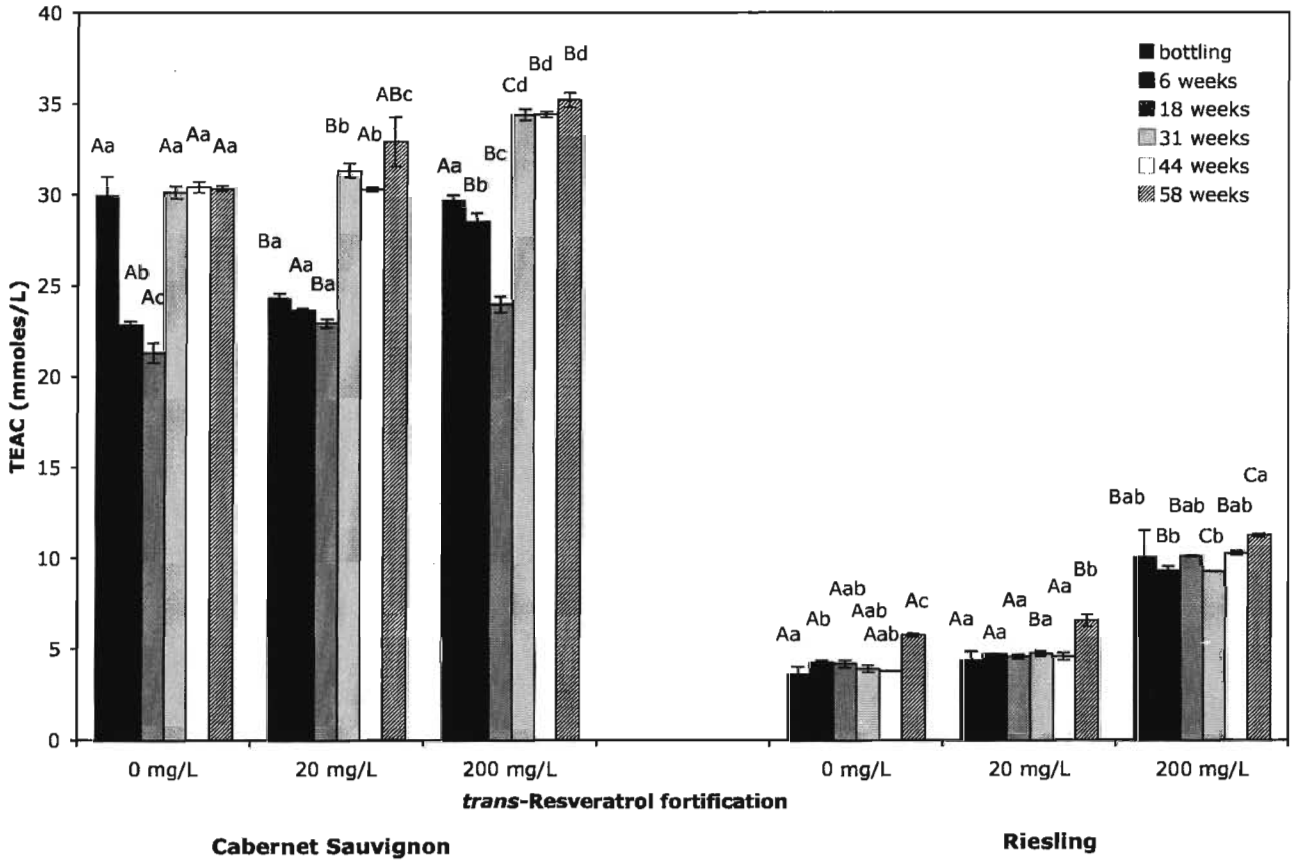

Figure 3.1. Anti-oxidative capacity [Trolox™ equivalent antioxidant capacity (TEAC) assay] in Cabernet Sauvignon and Riesling wines fortified with 0 mg/L, 20 mg/L, or 200 mg/L *trans*-resveratrol. Bars represent mean values of duplicate measurements ± standard deviations. Means with letters are those which differ significantly, and of those, means sharing the same letter do not differ in groups across time [lowercase] or at specific time points [uppercase] (Fisher's $LSD_{0.05}$).

Table 3.2. Basic spectrophotometric estimates of wine colour (WC) and phenolic composition for Cabernet Sauvignon wines. Data represent mean values of duplicate measurements from duplicate bottles ± standard deviations. Means sharing the same letter do not differ significantly across time [lowercase] or at a specific time point [uppercase] (Fisher's LSD $_{0.05}$).

Cabernet Sauvignon	Bottling	6 weeks	18 weeks	31 weeks	44 weeks	58 weeks
WC hue (A420 nm/A520nm)						
0 mg/L	0.633 Aa ± 0.000	0.648 Ab ± 0.005	0.711 Ac ± 0.001	0.738 Ad ± 0.006	0.766 Ae ± 0.003	0.796 Af ± 0.004
20 mg/L	0.625 Ba ± 0.002	0.632 Bb ± 0.010	0.713 Ac ± 0.002	0.731 Bd ± 0.003	0.763 Be ± 0.001	0.784 Bf ± 0.004
200 mg/L	0.592 Ca ± 0.002	0.597 Cb ± 0.004	0.682 Bc ± 0.001	0.705 Cd ± 0.002	0.740 Ce ± 0.002	0.750 Cf ± 0.003
WC density (A520 nm/A420 nm)						
0 mg/L	4.31 Aa ± 0.000	4.58 Ab ± 0.124	5.22 Ac ± 0.070	5.43 Ad ± 0.072	5.43 Ad ± 0.057	5.27 Ae ± 0.186
20 mg/L	4.19 Ba ± 0.007	4.48 Bb ± 0.013	5.52 Bc ± 0.017	5.67 Bd ± 0.028	5.65 Bd ± 0.015	5.57 Be ± 0.225
200 mg/L	4.39 Ca ± 0.007	4.88 Cb ± 0.145	5.70 Cc ± 0.005	6.01 Cd ± 0.087	5.92 Ce ± 0.029	5.67 Cc ± 0.189
Total red pigments (A520 nm/AHCl520 nm x 100)						
0 mg/L	14.6 Aa ± 0.431	13.3 Ab ± 0.344	13.7 Ac ± 0.266	13.1 Abd ± 0.127	12.7 Ad ± 0.082	10.8 Ae ± 0.126
20 mg/L	13.8 Aab ± 0.714	12.3 Bd ± 0.257	14.2 Aa ± 0.712	13.5 Ab ± 0.267	12.9 Ac ± 0.390	11.6 Be ± 0.586
200 mg/L	14.8 Aa ± 0.000	12.8 Bc ± 0.769	14.5 Aa ± 1.271	13.6 Ab ± 0.515	13.0 Abc ± 0.130	11.1 Cd ± 0.150
Degree of red pigment colouration (A520 nm/AHCl520 nm x 100)						
0 mg/L	18.0 Aa ± 0.531	20.9 Ab ± 0.048	22.2 Ac ± 0.162	23.9 Ad ± 0.292	24.1 Ad ± 0.348	27.2 Ae ± 0.746
20 mg/L	18.6 Aa ± 1.013	22.4 Bb ± 0.483	22.7 Ab ± 0.334	24.3 Ac ± 0.371	24.9 Ac ± 0.785	27.0 Ad ± 0.282
200 mg/L	18.7 Aa ± 0.048	24.0 Cb ± 0.881	23.5 Ab ± 1.980	25.9 Bc ± 0.810	26.2 Bc ± 0.341	29.1 Bd ± 0.652
Total phenolics (AHCl280 nm - 4)						
0 mg/L	21.1 Aa ± 1.15	20.6 Aa ± 0.629	24.8 Ab ± 0.529	25.8 Ac ± 0.379	27.2 Ad ± 0.240	22.2 Ae ± 0.377
20 mg/L	19.1 Aa ± 1.23	19.5 Ba ± 0.577	27.0 Ab ± 0.375	27.8 Bb ± 0.651	28.9 Bc ± 0.735	24.7 Bd ± 1.47
200 mg/L	30.9 Ba ± 0.219	28.9 Cb ± 2.03	37.3 Bc ± 2.36	38.8 Ccd ± 1.72	39.3 Cd ± 0.543	33.6 Ca ± 0.646

Table 3.3. Basic spectrophotometric estimates of phenolic composition for Riesling wines. Data represent mean values of duplicate measurements from duplicate bottles ± standard deviations. Means sharing the same letter do not differ significantly across time [lowercase] or at a specific time point [uppercase] (Fisher's LSD $_{0.05}$).

Riesling	Bottling	6 weeks	18 weeks	31 weeks	44 weeks	58 weeks
Total phenolics (A280 nm – 4)						
0 mg/L	0.041 Aa ± 0.014	0.453 Aa ± 0.019	2.36 Ab ± 0.042	2.76 Ac ± 0.058	3.59 Ad ± 0.116	3.41 Af ± 0.189
20 mg/L	1.67 Ba ± 0.028	1.56 Ba ± 0.041	3.40 Bb ± 0.134	4.22 Bc ± 0.057	4.86 Bd ± 0.064	4.31 Be ± 0.092
200 mg/L	12.3 Ca ± 0.014	11.2 Cb ± 0.161	12.9 Cc ± 0.131	14.5 Cd ± 0.067	15.6 Ce ± 0.070	14.5 Cd ± 0.112
Total hydroxycinnamates (A320 nm – 1.4)						
0 mg/L	0.115 Aa ± 0.014	0.068 Ab ± 0.019	1.38 Ac ± 0.042	1.43Ad ± 0.058	2.19 Ae ± 0.116	0.409 Af ± 0.038
20 mg/L	2.49 Ba ± 0.028	1.81 Bb ± 0.041	3.33 Bc ± 0.134	3.73 Bd ± 0.057	3.46 Be ± 0.064	3.58 Bf ± 0.056
200 mg/L	23.3 Ca ± 0.014	20.5 Cb ± .0161	22.2 Cc ± 0.131	23.9 Cd ± 0.067	14.2 Ce ± 0.070	23.5 Cf ± 0.128
Browning (A420 nm)						
0 mg/L	0.075 Aa ± 0.002	0.076 Aa ± 0.000	0.116 Ab ± 0.004	0.107 Ac ± 0.002	0.126 Ad ± 0.006	0.112 Ae ± 0.001
20 mg/L	0.079 Aa ± 0.000	0.083 Bb ± 0.001	0.110 Bc ± 0.005	0.121 Bd ± 0.008	0.132 Be ± 0.003	0.115 Bf ± 0.002
200 mg/L	0.075 Aa ± 0.000	0.094 Cb ± 0.003	0.122 Cc ± 0.003	0.131 Cd ± 0.004	0.142 Ce ± 0.001	0.129 Cf ± 0.001
Pinking (A520 nm)						
0 mg/L	0.013 Aa ± 0.002	0.012 Aa ± 0.001	0.034 Ab ± 0.004	0.028 Ac ± 0.002	0.034 Ab ± 0.002	0.022 Ad ± 0.000
20 mg/L	0.014 Aa ± 0.001	0.016 Bb ± 0.002	0.029 Bc ± 0.002	0.034 Bc ± 0.005	0.037 Ae ± 0.002	0.025 Bf ± 0.002
200 mg/L	0.014 Aa ± 0.001	0.020 Cb ± 0.002	0.038 Cc ± 0.001	0.038 Cc ± 0.002	0.043 Bd ± 0.001	0.034 Ce ± 0.001

Estimate of total hydroxycinnamate and phenolic concentrations were significantly higher in *t*R fortified Riesling (200 mg/L > 20 mg/L > control). This result is expected, as *t*R and *cis*-resveratrol are absorbed at 306 and 285 nm, respectively (Montsko et al., 2008). Estimates of browning and pinking increase over time for all treatments, and tend towards higher values with increasing *t*R fortification.

Resveratrol

Concentrations of *t*R in Riesling and Cabernet Sauvignon are shown in Figure 3.2. Due to the potential for polyphenols to self-associate and bind with other chemical constituents in wine (e.g. anthocyanins), we hypothesized that high levels of *t*R would result in the formation of precipitates – ultimately leading to a decrease in *t*R over time (Clarke and Bakker, 2004).

However, following a small initial drop in *t*R between bottling and 6 weeks at both fortification levels, *t*R is stable over for the remainder of the 58 week monitoring period. The initial decrease in *t*R concentration may be due to the known propensity of polyphenolic compounds to self-associate and complex with other wine constituents. *cis*-Resveratrol was detected in the 20 mg/L and 200 mg/L *t*R for Riesling and Cabernet Sauvignon treatments, with the highest concentration at 6 weeks post-bottling (± SD) (3.11 ± 0.18 mg/L, 6.42 ± 2.04 mg/L, 1.01 ± 0.06 mg/L, and 4.64 ± 0.33 mg/L for 20 mg/L and 200 mg/L Riesling and Cabernet Sauvignon, respectively).

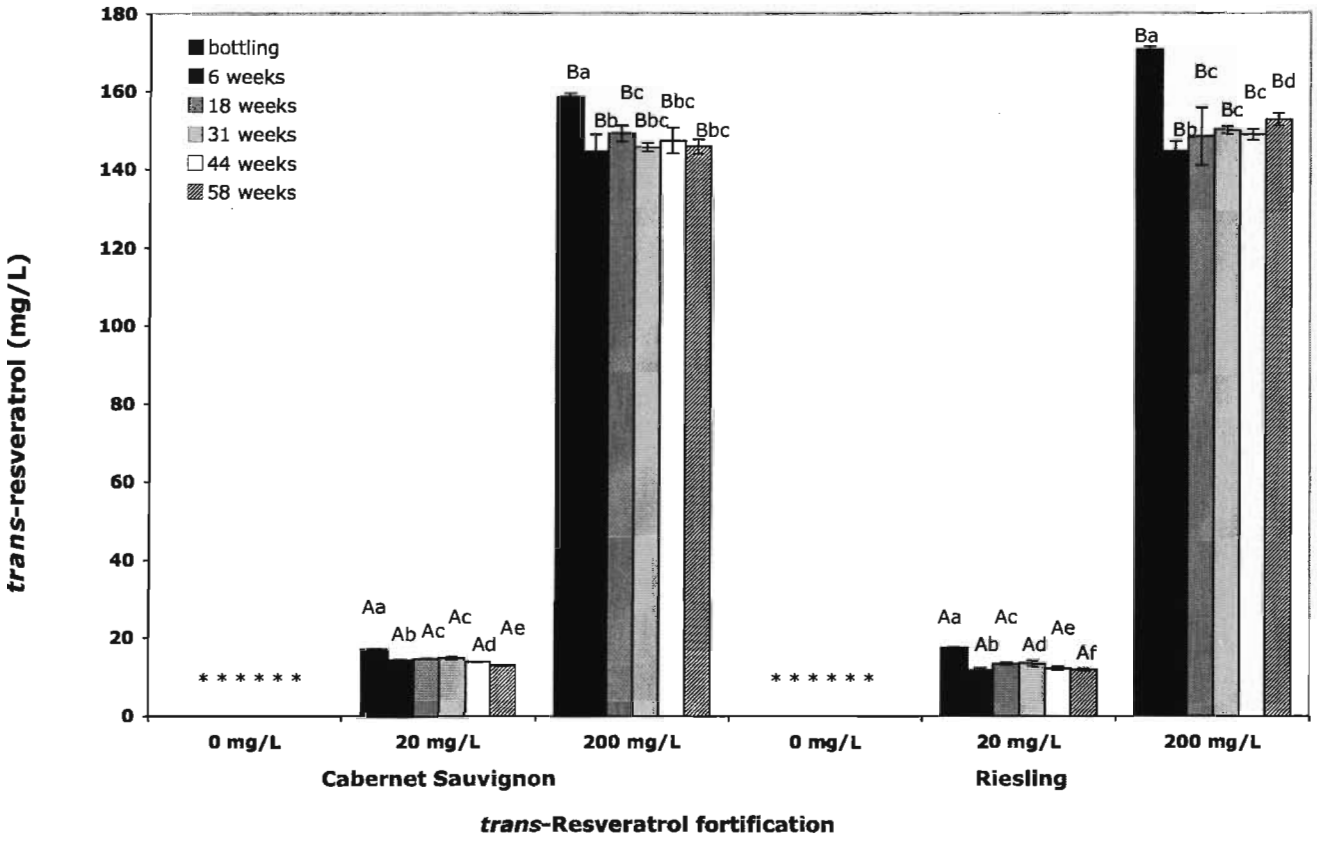

Figure 3.2. Concentration of *trans*-resveratrol in Cabernet Sauvignon and Riesling wines fortified with 0 mg/L, 20 mg/L, or 200 mg/L *trans*-resveratrol. 0 mg/L was below level of quantitation (0.493 mg/L). (Bars represent mean values of duplicate measurements of duplicate bottles ± standard deviations. Means with letters are those which differ significantly, and of those, means sharing the same letter do not differ in groups across time [lowercase] or at specific time points [uppercase] (Fisher's $LSD_{0.05}$). * refers to concentrations below the level of quantitation (0.493 mg/L).

Low concentrations of *cis*-resveratrol were detected at bottling in the 20 mg/L and 200 mg/L treatments in both varieties (0.00-0.56 mg/L), and reached their highest level 6 weeks post-bottling (1.009-6.415 mg/L). Following 6 weeks, *cis*-resveratrol decreased to below the limit of quantitation (LOQ) (0.134 mg/L) in Cabernet Sauvignon at most time points, and was substantially lower at the remaining time points in Riesling (data not shown).

Sensory analyses

Difference testing – triangle tests

We hypothesized that *t*R fortification would alter the flavour profile of Cabernet Sauvignon and Riesling wines. At 6 weeks, significant differences were found for 200 mg/L vs control Riesling ($p<0.01$). No differences were found for 20 mg/L vs control Riesling ($p<0.05$) or between Cabernet Sauvignon wines (0 mg/L vs 20 mg/L; 0 mg/L vs 200 mg/L; $p > 0.05$). A significant difference in colour was found for 200 mg/L vs control Cabernet Sauvignon ($p < 0.001$). No differences were found for the remainder of the Cabernet Sauvignon and Riesling treatments vs control.

At 32 weeks, significant differences were found for bitterness in Riesling (200 mg/L vs control; $p < 0.001$; 20 mg/L vs 200 mg/L; $p<0.01$). No differences were found for 20 mg/L vs control Riesling ($p > 0.05$). No differences were found for Cabernet Sauvignon wines (0 mg/L vs 20 mg/L; 0 mg/L vs 200 mg/L; $p > 0.05$). Significant differences in colour were found for 20 mg/L ($p \leq 0.05$) and 200 mg/L ($p \leq 0.001$) vs control Cabernet Sauvignon, and 200 mg/L vs control Riesling ($p < 0.05$).

Descriptive analysis

Results of descriptive analyses conducted on Riesling wines at 16 and 40 weeks are summarized in Figure 3.3. At 16 weeks (Figure 3.3A) 200 mg/L was rated higher in bitterness than 20 mg/L or control [($F(5,42)=16.92$, ($p<0.05$)]. Floral and vegetal aromas were rated higher for 20 mg/L compared to control [$F(5,31)=8.14$, ($p<0.05$) and ($F(5,24)=16.77$), ($p<0.05$) respectively]. Herbaceous notes have also been reported for white wines produced from grapes treated with ultraviolet - C light to increase resveratrol concentration (Guerrero et al., 2010). At 40 weeks (Figure 3.3B), the 200 mg/L treatment was rated higher than 20 mg/L [$F(9,73)=25.33$, ($p<0.01$)] and control [$F(9,73)=25.33$, ($p<0.001$)] for bitterness.

DISCUSSION AND CONCLUSIONS

For both Riesling and Cabernet Sauvignon wines, *t*R remains in solution for at least 58 weeks under normal cellar conditions, demonstrating a functional, shelf-stable product. Of interest, *cis*-resveratrol was detected at elevated concentrations at 6 weeks in all *t*R – fortified wines. The origin of the *cis* isomer at this time point is undetermined, and previous results on light-induced isomerization from *trans* to *cis* in wine and aqueous *t*R isolated from hops is conflicting (Jeandet et al., 1995a; Jerkovic and Collin, 2008).

Due to the association of polyphenols with anti-oxidant activity, we hypothesized that *t*R fortification would increase the anti-oxidant capacity of these wines. The results support this hypothesis and provide evidence that *t*R fortification, especially at higher

levels (20 mg/L < 200 mg/L), may provide additional anti-oxidant protection for wines during

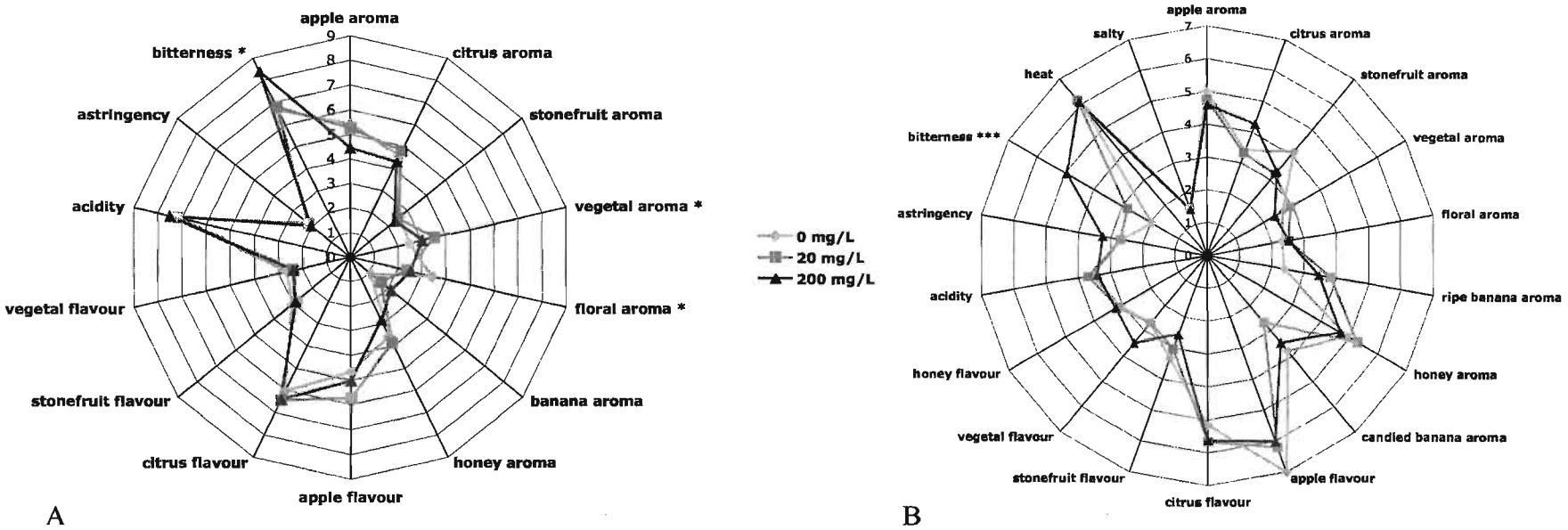

Figure 3.3. Cobweb diagrams representing mean intensity ratings for Riesling aroma and flavour at 16 weeks (A) and 40 weeks (B). Means from triplicate assessments, n=6 (A), and 10 (B). *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$ (Tukey's HSD).

storage. Whether this translates into biological or health-relevant benefits after consuming these wines remains to be determined.

Wine colour and phenolic results provide evidence of a colour shift for Cabernet Sauvignon with increasing *t*R fortification. These results are supported by visual difference tests performed at both 6 and 31 weeks. Such an effect may be explained by copigmentation; where wine anthocyanins associate with other non-coloured molecules, mostly phenolics, to create an enhancement in the absorbance and/or a bathochromic shift in wavelenth resulting in blue-purple tones (Boulton, 2001). At least seven different wine phenolics, including catechin and epicatechin, can associate with malvidin 3-glucoside, the major anthocyanin in red wine, leading to colour changes (Gomez-Miguez et al., 2006). *t*R may similarly bind with malvidin 3-glucoside and/or other wine anthocyanins. Other basic chemical indicators of wine quality, including TA, pH, and SO_2, were similar in *t*R fortified and control wines.

*t*R fortification did not alter the flavour or aroma profile of Cabernet Sauvignon. Although it is possible that the use of oak masked any flavour changes attributable to *tR* fortification, we speculate that the higher intrinsic bitterness typical of wine made from this variety would make any bitterness-eliciting properties of *t*R harder to perceive. However, these findings may not apply to other red varieties, especially those that do not typically elicit high levels of bitterness, such as Pinot Noir and Gamay. Bitterness was perceived as significantly more intense in 200 mg/L *t*R Riesling. Because of their naturally lower phenolic content and the minimal skin contact employed during vinification, wine made from white grapes does not typically elicit much bitterness; thus the contribution from *t*R may be more easily perceived in this style. Thus, further flavour

modification and/or lower fortification levels may be desirable in *t*R - fortified white wines.

While *t*R fortification may be successful for some wine styles, there are some significant limitations to its entry into the current market. Present legislation in most wine jurisdictions does not allow direct fortification of wine with *t*R, although nomenclature such as 'wine-based beverage' or similar labeling is often permitted. Additionally, health claims typically cannot be made on wine labels, which reduces marketing opportunities for this product. However, there is significant potential for success for *t*R enriched wines, given the quality of the product as determined in this study, and the growing health conscious market (Barreiro-Hurlé et al., 2008). Further research on the fortification of other beverages and food with *t*R, as well as the enrichment of wine with other health-promoting wine polyphenols is proceeding.

Chapter 4 - THE EFFICACY OF BITTER BLOCKERS ON HEALTH-RELEVANT BITTERANTS

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The candidate is the primary author and contributor of this chapter. Dr. Gary Pickering has provided various edits throughout its development.

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INTRODUCTION

Food and beverage fortification with plant-based ingredients is an increasing trend in the functional food industry. Examples include flavonoid-enhanced chocolate bars and drink supplements, yogurt fortified with green tea phenolics, and grape phenolic additions to water, wine and bread (Gaudette and Pickering, 2011a). Of the numerous plant based-compounds that are currently being used as fortifying agents in functional foods, green tea extracts and grape-derived compounds are of particular value due to their associated health benefits.

Catechins, derived from both green tea (Mukhtar and Ahmad, 2000) and red wine (Waterhouse, 2002), are a family of polyphenols associated with chemopreventive effects on colon (Larsen et al., 2009), lung (Sadava et al., 2007), prostate (Bettuzzi et al., 2006), and breast (Damianaki et al., 2000) cancers. They are also associated with numerous cardiovascular health benefits, such as decreased inflammation (Norata et al., 2007), increased endothelial nitric oxide activity (Fisher et al., 2003), and improved blood lipid profile (Inami et al, 2007). Caffeine, another plant-derived ingredient, is increasingly being used in functional beverages, including energy drinks (Scholey and Kennedy, 2004; Tamamoto et al., 2010). It has a wide range of positive physical and physiological effects, including an increase in energy expenditure, alertness and wakefulness, an enhancement of short-term memory, cognitive function and neuromuscular coordination, and an increase in the ability to concentrate and focus attention (Glade, 2010; Heckman et al., 2010).

Although the addition of these compounds can increase the functionality of a product, caffeine (Leach and Noble, 1986) and polyphenols, including (+)-catechin

(Peleg et al., 1999) and *trans*-resveratrol (Gaudette and Pickering, 2011b), can elicit significant levels of bitterness, which are generally associated with reduced consumer acceptance of food and beverages (Lesschaeve and Noble, 2005). Thus, strategies to decrease bitterness in functional food formulations are timely and of considerable interest.

Using tastants to mask bitterness is a common strategy employed to improve the sensory profile and consumer acceptability of conventional foods. However, their use within functional foods can be problematic. For instance, the addition of sucrose and sodium chloride (Gaudette and Pickering, 2011a), are not optimal as they may be perceived to decrease the 'healthiness' of the product. Alternatives have included the use of carboxymethyl cellulose to decrease the bitterness of caffeine (Pangborn et al., 1973, 1979), and β-cyclodextrin (Shaw and Wilson, 1983) to lower the bitterness of naringin. Homoeriodictyol sodium salt (HED) has also been shown to reduce bitterness across an array of bitterants, including caffeine, quinine, denatonium benzoate, salicin, guaifenesin, paracetamol and amarogentin (Ley et al., 2005), and may be particularly attractive for use in functional food formulations. In conjunction with other flavanones extracted from the Herba Santa shrub (*Eriodictyon californicum*) (Geissman, 1940), homoeriodictyol has been historically used to mask the bitterness of quinine, and to treat colds and asthma. However, it can elicit a strong aroma, and thus its sodium salt (also an extract of Herba Santa) may be of greater value as it can also reduce bitterness while eliciting minimal side tastes or aroma (Ley et al., 2005).

In addition, zinc and magnesium sulfate have been used to decrease the bitterness of various pharmaceuticals (Keast, 2003; Keast and Breslin, 2005). The modes of action

whereby these alternative compounds reduce bitterness vary: complexation or encapsulation of bitterants; interaction with bitter binding sites on taste receptor cells; formation of a physical barrier between bitterant and taste receptor; or perhaps through interference with taste transduction mechanisms further downstream (Gaudette and Pickering, 2011a).

There is evidence that differences exist in the bitter blocking capacity of some of these compounds, which may be due to their different modes of action. For instance, compared to magnesium sulfate, zinc sulfate is significantly more effective at lowering the perceived bitterness of quinine hydrochloride [52% and 70% decrease, respectively (Keast, 2003)]. With nearly 30 different families of TAS2R bitter taste receptors, and evidence of bitterants sharing the same receptors and/or transduction mechanisms (Delwiche et al., 2001; Keast & Breslin, 2002; Meyerhof et al., 2010), it is likely that bitter blockers that target specific portions of the extracellular component of taste receptors will differ in their capacity to reduce bitterness. Similar differences may exist for bitter blockers that encapsulate bitterants, as intermolecular binding of blocker and bitterant is likely based on specific binding affinities. Given these differences in the performance of bitter blockers, it is also of value to assess their relative effectiveness on health-promoting, plant-based bitterants.

We hypothesise that the effectiveness of bitter blockers is dependent on both the bitterant and the blocker's mode of action, and we investigate this using two plant-derived bitterants from different chemical families. The main objective of this study is to determine the efficacy of a range of bitter blockers on the bitterness elicited by (+)-

catechin, a polyphenol, and caffeine, a xanthine alkaloid, in aqueous solutions. While some studies have examined the effectiveness of some blockers on reducing the bitterness of caffeine (Ley et al., 2005; Keast, 2008), our work will expand on this by including additional bitter blockers, and will include (+)-catechin so that a comparison of bitter blocking efficacy between these two bitterants can be made. The capacity of these bitter blockers to affect other orosensory sensations is also examined; this information will assist in determining their suitability for application in functional food formulations.

MATERIAL AND METHODS

Panelists

Panelists (n = 12, 32 ± 10 years old, 6 males) between the ages of 21 and 52, were staff or students of Brock University's Cool Climate Oenology and Viticulture Institute (CCOVI). All panelists volunteered their time and signed consent forms that had been cleared by Brock University's Research Ethics Board. All training and data collection sessions were held in CCOVI's sensory evaluation lab.

Panelist screening and training

Screening and training of panelists involved correctly identifying and rating the intensity of six aqueous solutions, each containing a taste (sweet, sour, salty, bitter, umami) or tactile (astringent) stimulus. In order to familiarize panelists with scale usage,

intensity ratings were recorded on a 15 cm line scale with 1 cm indented anchors (bottom anchor of 'absent', top anchor of 'high'). Panelists were first presented a single flight of solutions in International Organization for Standardization (ISO) wine tasting glasses. Each glass contained 20 mL of a single stimulus, and was labeled with the appropriate taste or tactile sensation. Instructions for tasting and rating were the following: *take the full amount of solution into the mouth, swirl for 5 seconds, expectorate the sample, wait at least 5 seconds for oral sensation to fully develop, rate the highest intensity experienced for the oral sensation on the line scale, rinse thoroughly 4 times with water, wait a minimum of 1 minute between samples.* During a mandatory 10-minute break that followed, panelists completed various questionnaires related to demographics and food and beverage behaviour. After this, a second flight of the same solutions was randomly presented in glasses labeled with 3-digit, randomized codes. The same instructions for tasting and rating were followed, and in addition, panelists were asked to correctly identify the taste or tactile stimulus in each glass.

Screening and training were considered completed when panelists correctly identified and rated each stimulus presented in the second flight. An error in correctly identifying any stimulus resulted in the panelist being invited back for an additional training and screening session. If an error in identification occurred during this additional session, panelists were thanked and excused from participating in the remainder of the study (n=1).

Stimuli

Chemicals and concentrations used for the screening and training for basic tastes and tactile sensation were based on previous values from the literature (Keast, 2003; Pickering et al., 2006) and consisted of sucrose (sweetness, 3.0×10^{-1} M), citric acid (sourness, 3.0×10^{-3} M), sodium chloride (saltiness, 1.5×10^{-1} M), L-glutamic acid monosodium salt hydrate (umami, 1.0×10^{-1} M), quinine monohydrochloride dihydrate (bitterness, 5.0×10^{-5} M), and aluminum sulfate (astringency, 4.4×10^{-4} M).

The bitterants used in the data collection sessions were caffeine and (+)-catechin hydrate, presented at either low or high concentrations, while treatments consisted of one of five bitter-blocking compounds, presented at low or high concentrations (Table 4.1). All chemicals for screening, training and data collection were sourced from Sigma-Aldrich (Oakville, ON, Canada), with the exception of HED (Symrise, Holzminden, Germany). Initial stimulus concentrations for the bitterants and bitter blockers were sourced from various literature values, and modified as needed through bench testing (n=7, data not shown) to emulate levels more appropriate for potential use in functional food formulations (Table 4.1).

Table 4.1. Bitterants and bitter blockers used in study.

Bitterant – functional ingredient	**Low concentration**	**High concentration**
Caffeine	4.5×10^{-3} M	1.1×10^{-2} M
(+)-Catechin	3.4×10^{-3} M	6.2×10^{-3} M
Bitter blocker		
Zinc sulfate monohydrate	3.1×10^{-3} M	6.3×10^{-3} M
Magnesium sulfate	2.5×10^{-2} M	5.0×10^{-2} M
β-cyclodextrin	0.3% (w/v)	0.6% (w/v)
Carboxymethylcellulose sodium salt – low viscosity	0.6% (w/v)	1.2% (w/v)
Homoeriodictyol sodium salt	3.1×10^{-4} M	6.2×10^{-4} M

Sample preparation

All solutions were prepared using fresh deionized water (Millipore RiOs 16 Reverse Osmosis System, Bedford, MA, USA). 1 mL of 99% pure food grade ethanol (Liquor Control Board of Ontario, St. Catharines, ON, Canada) was used to fully solubilize (+)-catechin prior to its addition to solutions made in 100 mL volumetric flasks. This concentration of ethanol is below perception threshold in water (Thorngate and Noble, 1995). Samples were prepared in foil-wrapped volumetric flasks to protect (+)-catechin from possible light-induced polymerization (Peleg et al., 1999). Samples were then transferred to airtight, 120 mL amber coloured glass bottles (Fisher Scientific, Rockford, IL, USA). The headspace was filled with nitrogen gas, and samples stored in darkness at 4°C and replaced every 5 days.

To determine whether polymerization of (+)-catechin would occur following sample preparation and storage, reversed-phase high performance liquid chromatography (HPLC) was performed (Ibern-Gomez et al., 2002). A Hewlett-Packard (HP) series 1100 gradient UV/Vis liquid chromatograph with diode-array detector, corresponding HP ChemStation for LC software (Rev.A.07.01 [682]), and an Agilent (Agilent Technologies, Santa Clara, CA, USA) 50 x 4.6 mm, 3.5 μm Zorbax SB-C18 column at 30°C was used for detection and analysis. On each day, for five consecutive days, solutions were prepared and stored until HPLC analysis was performed on the final day. Daily solution preparation involved solubulizing 0.12 g of (+)-catechin hydrate in 1 mL of 95% HPLC grade ethanol (Fisher Scientific, Rockford, IL, USA). The solubulized (+)-catechin was then transferred to a 100 mL foil-wrapped volumetric flask, and brought up to volume using pure water (Millipore RiOs 16 Reverse Osmosis System, Millipore Corporation, Bedford, MA, USA). Samples were immediately transferred to 2 mL amber, snap top chromatography vials (Agilent Technologies) and stored at 4°C until analysis. Over the course of this 5 day time period, (+)-catechin remained in monomeric form. No additional peaks appeared on the chromatogram, retention time remained the same, and there was no significant change in peak area (data not shown).

Design

A randomized block design was used for this experiment. All possible combinations of bitter blockers at both low and high concentrations were paired with each of the bitterants at both low and high concentrations. Thus, 40 different binary solutions plus four controls [low and high concentrations of caffeine and (+)-catechin]

were presented to all panelists, in duplicate. The 88 samples were presented over 11 separate sessions. The duplicate assessment of samples took place once the first assessment was completed, and these were also presented in randomized order. A session consisted of two flights of four glasses each. Forced breaks of 3 and 10 minutes, along with four water rinses, were enforced between samples and flights, respectively. Samples were removed from the refrigerator 1 hour prior to testing. 20 mL of each sample were then poured into black tasting glasses (Spiegelau, Edison, NJ, USA) to mask any visual differences and labeled with randomized 3-digit numbers.

Panelist responses were collected using a computerized program (Compusense, Guelph, ON, Canada). They were presented on the same screen with seven separate, 15 cm line scales with 1 cm indented anchors (bottom anchor of 'absent', top anchor of 'high'). Each sample was rated for intensity of bitterness, astringency, sweetness, sourness, saltiness, savouriness, and 'other' – a term which was used to capture any additional oral sensations that may have been elicited. Nose-clips were worn to decrease possible retronasal influences.

For each sample, the following tasting protocol was strictly adhered to: *apply nose clip, take the full solution into the mouth, swirl in mouth for 5 seconds, expectorate the sample, wait 10 seconds for oral sensations to fully develop* (panelists were verbally instructed to wait longer if intensity was still building after 10 seconds), *rate the highest intensity experienced for each oral sensation, remove nose clip, rinse thoroughly with water at least 4 times during the forced 3 minute break, continue onto the next sample only when mouth is completely free from all taste and tactile sensations.*

Statistical analyses

Statistical analysis was performed using XLSTAT version 2011.1.01 for Apple Macintosh (Addinsoft, New York, NY, USA). Initial analyses were performed to determine individual panelist performance and to identify possible outliers. Three criteria were used to define an outlier for bitterness responses: (1) Reproducibility between reps. Coefficient of variation between each duplicate measurement, averaged across x treatments > 100%. (2) Bitterness sensitivity. Intensity ratings < 1.0 cm average across both replicates. (3) Discrimination. $p(F) > 0.05$ for bitter blocker treatment from 2-way ANOVA (rep and treatment as independent variables).

If a panelist met two or more of these three criteria, they were identified as outliers and removed from the dataset (n=2). Three-way ANOVAs were then conducted for bitterness and astringency, with analyses performed separately for both high and low concentrations of (+)-catechin and caffeine. For each ANOVA, the dependent variable was the difference between the intensity rating of the control solution (e.g. caffeine – low concentration) and the rating for the corresponding bitter blocker. In calculating these values, the duplicate ratings of each panelist were averaged. The ANOVA model included bitter blocker, bitter blocker concentration, panelist, and all 2-way interactions as independent variables in each initial analysis. Analysis was repeated with panelist x bitter blocker concentration and/or panelist x bitter blocker interaction(s) removed if these terms were not significant ($p(F)<0.05$). Tukey's HSD mean separation tests were used for post-hoc analyses ($\alpha = 0.05$).

Two-way ANOVAs were conducted for sweetness, sourness, savoriness, and 'other', with analyses performed separately for both high and low concentrations of (+)-

catechin and caffeine. For each ANOVA, the dependent variable was the intensity rating for the control solution or bitter blocker treatment. In calculating these values, the duplicate ratings of each panelist were averaged. The ANOVA model included panelist, bitter blocker and bitter blocker x panelist interaction as independent variables in the initial analysis. Analysis was repeated with the interaction term removed if not significant ($p(F)<0.05$). Tukey's HSD mean separation tests were used for post-hoc analyses ($\alpha = 0.05$).

2-Sample t-tests were also conducted for control versus high and control versus low bitter blocker concentration for both bitterness and astringency responses ($\alpha = 0.10$, 0.05).

RESULTS

Main results for bitterness and astringency from each bitter blocker treatment are expressed as the difference in bitterness or astringency intensity rating from control (e.g., (+)-catechin and/or caffeine response minus bitter blocker treatment response). Therefore, higher values infer a stronger bitterness or astringency lowering effect.

Effect on bitterness of (+)-catechin

Results show significant differences in the capacity for bitter blockers to decrease the bitterness of both high and low concentrations of (+)-catechin (Figure 4.1). For both high and low (+)-catechin concentrations, 3-way ANOVA results reveal a main effect for

bitter blocker [($F(4,45) = 9.8$, ($p<0.0001$); ($F(4,45) = 6.6$, $p<0.0001$), respectively] and panelist [($F(9,45) = 21.9$, $p < 0.0001$; ($F(9,45) = 18.3$, $p<0.0001$, respectively) and a significant interaction for bitter blocker x panelist [($F(36,45) = 2.1$, $p = 0.01$; ($F(36,45) = 2.1$, $p = 0.01$), respectively]. There was no main effect for bitter blocker concentration and no significant interaction for bitter blocker x concentration ($p > 0.05$).

β-CYCLO was the most effective bitter blocker for decreasing the bitterness of both high and low concentrations of (+)-catechin (Figure 4.1), with mean intensity differences in bitterness from control of 3.6 and 1.9 cm, respectively. In both instances, this is a decrease in bitterness of approximately 60%.

Both the high and low concentrations of HED decreased the bitterness of (+)-catechin, although only for the high (+)-catechin concentration, where the reduction compared to control was 33%. With the exception of β-CYCLO, the bitter blocking capacity of HED is not significantly different from other bitter blocker treatments (Figure 4.1).

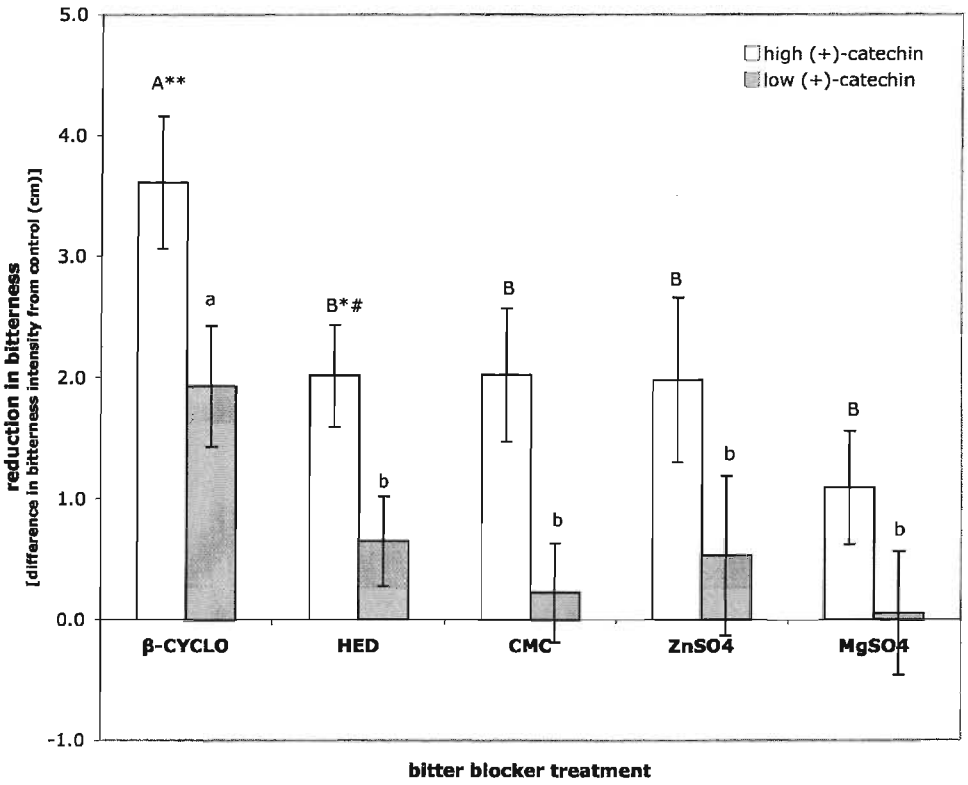

Figure 4.1. Effectiveness of bitter blockers in reducing bitterness of high and low concentrations of (+)-catetchin (6.2 x 10^{-3} and 3.4 x 10^{-3} M, respectively) using a 15 cm line scale. Values shown represent mean responses from panelists (n=10), pooled across rep, ± SEM. Means sharing the same letter do not differ significantly across bitter blockers for high (+)-catechin (uppercase) and low (+)-catechin (lowercase) (Tukey's $HSD_{0.05}$). 2 sample t-test results comparing control bitterant response vs high or low bitter blocker concentrations are denoted by: ** = high bitter blocker concentration $p<0.05$, * = high bitter blocker concentration $p<0.10$, # = low bitter blocker concentration $p<0.10$. Average control response for high (+)-catechin = 6.13, for low (+)-catechin = 3.23. Bitter blockers with corresponding high and low concentrations: β-CYCLO (β-cyclodextrin; 0.3%, 0.6%); HED (homoeriodictyol sodium salt; 6.2 x 10^{-4}, 3.1 x 10^{-4} M); CMC (carboxymethylcellulose sodium salt – low viscosity; 1.2%, 0.6%); $ZnSO_4$ (zinc sulfate monohydrate; 6.3 x 10^{-3}, 3.1 x 10^{-3} M); $MgSO_4$ (magnesium sulfate; 5.0 x 10^{-2}, 2.5 x 10^{-2} M).

However, for some functional food and beverage formulations, HED may be a more optimal blocker than others (e.g., CMC and $ZnSO_4$) due to the minimal elicitation of other side tastes and sensations.

The remaining blockers - CMC, ZnSO4 and MgSO4 - showed similar bitterness reduction to HED for the high concentration of (+)-catechin, and minimal effect for the low concentration, although none of these results were statistically different from their controls.

Effect on bitterness of caffeine

Results show significant differences in the capacity for various bitter blockers to affect the bitterness of both high and low concentrations of caffeine (Figure 4.2). For both high and low caffeine concentrations, 3-way ANOVA results show a main effect for bitter blocker [$F(4,36) = 16.2$, $p < 0.0001$; $F(4,36) = 16.5$, $p < 0.0001$), respectively] and panelist [$F(9,36) = 14.3$, $p < 0.0001$; $F(9,36) = 11.9$, $p < 0.0001$), respectively] and a significant interaction for bitter blocker x panelist for high caffeine ($F(36,36) = 4.9$, $p < 0.0001$) and low caffeine ($F(36,36) = 2.1$, $p < 0.05$) concentrations. For high caffeine only, there was a main effect for bitter blocker concentration ($F(1,36) = 7.6$, $p < 0.01$), but no significant interaction for bitter blocker x concentration for either high or low caffeine ($p > 0.05$).

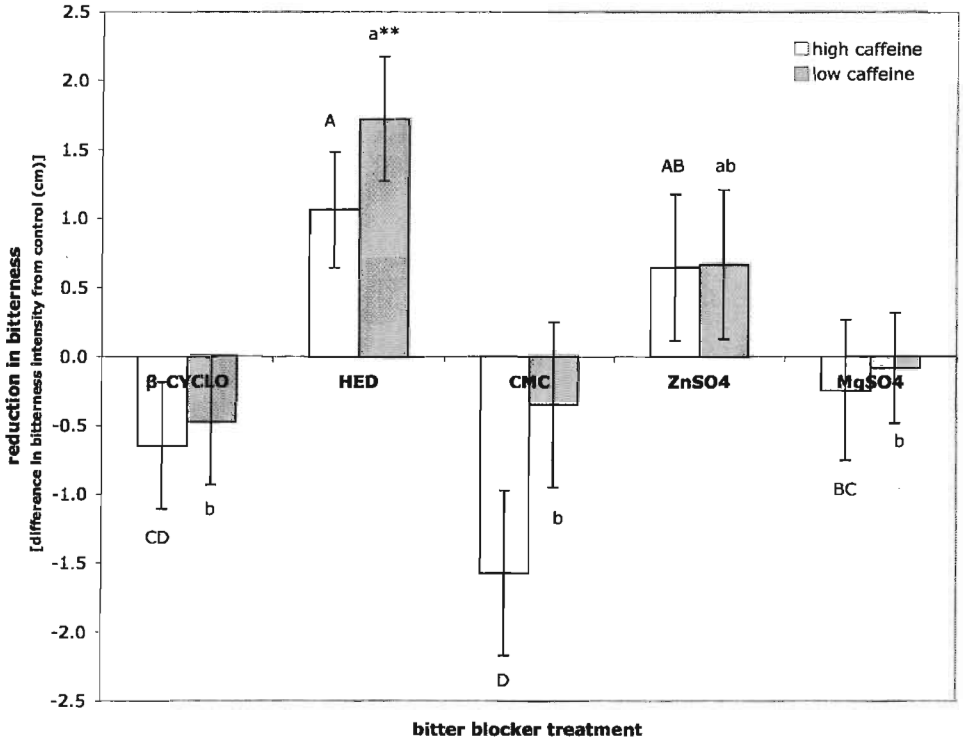

Figure 4.2. Perceived difference in bitterness intensity from control of various bitter blockers paired with high and low concentrations of caffeine (1.1×10^{-2} and 4.5×10^{-3}, respectively) using a 15 cm line scale. Values shown represent mean responses from panelists (n=10), pooled across rep, ± SEM. Means sharing the same letter do not differ significantly across bitter blocker treatments for high caffeine (uppercase) and low caffeine (lowercase) (Tukey's $HSD_{0.05}$). 2 sample t-test results comparing control bitterant response vs high and low bitter blocker treatments are denoted by: ** = high bitter blocker concentration $p < 0.05$. Average control response for high caffeine = 7.12, for low caffeine = 4.05. Bitter blockers with corresponding high and low concentrations: β-CYCLO (β-cyclodextrin; 0.3%, 0.6%); HED (homoeriodictyol sodium salt; 6.2×10^{-4}, 3.1×10^{-4} M); CMC (carboxymethylcellulose sodium salt – low viscosity; 1.2%, 0.6%); $ZnSO_4$ (zinc sulfate monohydrate; 6.3×10^{-3}, 3.1×10^{-3} M); $MgSO_4$ (magnesium sulfate; 5.0×10^{-2}, 2.5×10^{-2} M).

HED was the most effective bitter blocker in decreasing the bitterness of both high and low concentrations of caffeine, although this was statistically different from control for the high bitter blocker and low caffeine concentration combination only (Figure 4.2). HED reduced the bitterness of high and low caffeine by 15% and 43%, respectively. Also, reductions of 9% and 16% resulted for $ZnSO_4$ in high and low caffeine solutions, respectively. The remaining blockers did not significantly affect bitterness ratings, although interestingly, they trend towards an increase in bitterness.

Other sensations

Results show significant differences in the capacity for various bitter blockers to alter the perceived astringency of (+)-catechin (Table 4.2). For both high and low (+)-catechin concentrations, 3-way ANOVA results show a main effect for bitter blocker [$F(4,36) = 102.5$, $p<0.0001$; $F(4,36) = 164.0$, $p<0.0001$, respectively], panelist [$F(9,36) = 19.4$, $p<0.0001$; $F(9,36) = 19.8$, $p<0.0001$, respectively] and a significant interaction for bitter blocker x panelist [$F(36,36) = 4.4$, $p < 0.0001$; $F(36,36) = 5.7$, $p<0.0001$, respectively]. There was a significant interaction for bitter blocker x concentration for high (+)-catechin only ($F(4,36) = 13.1$, $p < 0.0001$).

CMC and HED were the most effective bitter blockers for decreasing the astringency of both high and low concentrations of (+)-catechin. CMC decreased the astringency of high and low (+)-catechin by 34% and 44%, respectively. Also, HED decreased the astringency of high and low (+)-catechin by 17% and 33%, respectively. Other blockers were significantly less able to decrease the astringency of (+)-catechin, and in the case of $ZnSO_4$, a significant increase in astringency is observed at both

concentrations of (+)-catechin. $ZnSO_4$ also elicited significantly higher levels of astringency with both high and low concentrations of caffeine (-4.4 and -4.5 cm, respectively). However, no changes in astringency were found for any other caffeine treatment; an expected result, given that

Table 4.2. Impact of bitter blockers on the astringency of high and low concentrations of (+)-catechin. Responses from panelists (n=10) are expressed as mean difference in astringency intensity rating from control. For each concentration of (+)-catechin, bitter blocker means sharing the same letter do not differ significantly (Tukey's $HSD_{0.05}$).

Bitter blocker[a]	High (+)-catechin Difference from control[b] (cm)	Low (+)-catechin Difference from control[c] (cm)
β-CYCLO	-1.1c	0.30bc
HED	0.40ab	0.80ab
CMC	0.90a	1.1a
$ZnSO_4$	-5.0d	-4.7d
$MgSO_4$	-0.020b	0.00c

[a] response averaged across bitter blocker concentrations [β-CYCLO (β-cyclodextrin; 0.3%, 0.6%); HED (homoeriodictyol sodium salt; 6.2×10^{-4}, 3.1×10^{-4} M); CMC (carboxymethylcellulose sodium salt – low viscosity; 1.2%, 0.6%); $ZnSO_4$ (zinc sulfate monohydrate; 6.3×10^{-3}, 3.1×10^{-3} M); $MgSO_4$ (magnesium sulfate; 5.0×10^{-2}, 2.5×10^{-2} M)].

[b] average control response for high (+)-catechin = 2.53

[c] average control response for low (+)-catechin = 2.54

caffeine is not known to elicit significant astringency at the concentrations used here (data not shown).

Overall, the majority of bitter blockers at both high and low concentrations elicited no or low intensities of side tastes and other sensations. The mean and range of intensity responses (cms) for each oral sensation, pooled across replicate and bitterant, were: sweet (0.83; 0.20 – 2.3), sour (0.15; 0.0 – 0.72), salty (0.26; 0.0 – 1.2), savory (0.69; 0.0 – 3.4),'other' (0.88; 0.11 – 4.4). Compared with other sensations, higher maximum intensity ratings were given for savory (3.4) and 'other' (4.4). These ratings are due to the greater savory and viscosity scores elicited by ZnSO4 and CMC, respectively. A relatively high sweetness rating was also evident for the high concentration of β-CYCLO when paired with low caffeine (2.3).

DISCUSSION

The results from this study suggest that the effectiveness of bitter blockers on decreasing bitterness is bitterant dependent. For instance, β-CYCLO and HED significantly decreased the bitterness of (+)-catechin, while only HED decreased the bitterness of caffeine. It is important to note that these two bitter blockers differ in their mode of action. Bitter eliciting compounds interact with the interior of cyclodextrins, forming an inclusion complex that prevents the bitterant from binding to taste receptor cells (Szejtli, 1988). In contrast, it is hypothesized that HED targets specific extracellular sites on TAS2Rs (Ley et al., 2005). Different modes of action may be the primary reason for the differences in bitter blocking effectiveness of HED and β-CYCLO on caffeine and (+)-catechin. For instance, β-CYCLO may be ineffective at lowering the bitterness of

caffeine and chemically similar bitterants due to an inability to form an inclusion complex. However, HED may confer a bitter blocking effect by interacting with caffeine-binding TAS2Rs.

The difference in bitterness response between (+)-catechin and caffeine after treatment with β-CYCLO may partly be due to the use of a β-CYCLO concentration that is insufficient in effectively decreasing the bitterness of caffeine. Alternatively, β-CYCLO may be unable to adequately encapsulate caffeine. This latter suggestion is supported by previous use of cyclodextrins in decaffeination trials, where differences in the effectiveness of decaffeination were seen between polymerized and monomeric β-cyclodextrin, possibly due to an inability of the monomeric form to create an insoluble complex with caffeine (Yu, 1988). Thus, polymerized β-cyclodextrin may be more suitable for decreasing the bitterness of caffeine in functional food formulations.

It is likely that bitter blockers have an optimal concentration where they provide maximum bitterness reduction and minimal elicitation of undesirable side tastes and sensations. For instance, our results indicate that the bitterness perception of bitter blocker-high caffeine binary solutions is dependent on bitter blocker concentration; on average, higher concentrations of bitter blockers are not associated with greater bitterness inhibition. Additionally, $ZnSO_4$ increased the astringency intensity of both high and low (+)-catechin concentrations by approximately 200%. While $ZnSO_4$ has previously been reported to elicit astringency (Keast, 2003), it can also decrease the bitterness of an array of pharmaceuticals (Keast and Breslin, 2005). However, we caution against its use in

simple matrices that do not contain compounds that can mask the astringency elicited by $ZnSO_4$.

CMC was the most effective at decreasing the astringency of high and low (+)-catechin (reduction by 34 and 44%, respectively). Others report the ability of CMC to reduce the astringency elicited by phenolics, and that the level of reduction is dependent on CMC concentration (Troszyńska et al., 2010). The mode of action here is unclear, but may include the binding of phenolics with polysaccharides in place of salivary proteins, leading to a decrease in the perceived friction that is typically imparted by their complexation. In addition, the perceived drying or puckering sensations generally elicited by phenolics may be reduced by the perceived viscosity of polysaccharides (Smith et al., 1996; Troszyńska et al., 2010). While the use of higher concentrations of CMC may lead to an enhanced suppression of astringency, CMC also elicits significant viscosity, which may not be optimal in some functional food formulations.

Sweetness was elicited at a higher intensity by β-CYCLO paired with low caffeine compared to other bitter blocker-bitterant combinations. β-CYCLO has a recognition threshold of 0.11% (Toda et al., 1981). Therefore, in addition to the possible inability of β-CYCLO to effectively encapsulate caffeine, the high concentration used in this study (0.6%) likely elicited sweetness that was unable to be masked by the bitterness of free, non-encapsulated caffeine at a low concentration. All of these considerations suggest that optimum bitter blocker concentrations should be established when formulating functional food products; further research that seeks to model sensory responses across a greater range of stimuli, matrices, and blocker concentrations may assist in this regard.

Finally, we acknowledge that gross variation exists among individuals in the ability to perceive bitterness. While we did not quantify this possible variation in the present study, it may be of value to determine whether the efficacy of bitter blockers on bitterness perception varies with individual variation in taste perception. Methods used to predict perceived taste intensities have often been based on determining one's genetically influenced responsiveness to the bitterant 6-*n*-propylthiouracil (PROP) (Bartoshuk et al., 1994). Thus, based on one's sensitivity to PROP, these findings may not be fully representative of some sub-populations of individuals, particularly those who perceive these bitterants at high intensities. Future work may benefit from introducing methods such as these on a larger population in order to better understand the different effects bitter blockers may have on the bitterness perception of individuals with varying taste sensitivity.

CONCLUSION

The use of bitter blockers to decrease the bitterness of various health-promoting, plant-derived, functional ingredients is promising. While β-CYCLO is widely used throughout the food industry, this is the first study to provide evidence of its effectiveness in decreasing the bitterness of (+)-catechin – a functional ingredient used in numerous products. The novel compound HED is also effective at decreasing the bitterness of both (+)-catechin and caffeine, and may also decrease the astringency of (+)-catechin. HED may have significant value in functional food formulations based on naturally sourced ingredients, as it is herb-derived and elicits no side tastes.

Chapter 5 - OPTIMIZING THE OROSENSORY PROPERTIES OF FUNCTIONAL FOOD AND BEVERAGES: THE INFLUENCE OF NOVEL SWEETENERS, ODOURANTS, BITTER BLOCKERS AND THEIR MIXTURES ON (+)-CATECHIN

Nicole J. Gaudette and Gary J. Pickering

The candidate is the primary author and contributor to this chapter. Dr. Gary Pickering has provided various edits throughout its development.

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INTRODUCTION

The significant health benefits of grape and green tea-derived compounds have led to their use as fortifying agents in functional food and beverage formulations. For example, fortified yogurts, drink supplements, health bars, bread, and wine are now available on the market (Gaudette and Pickering, 2011a). Catechins, derived from both green tea (Mukhtar and Ahmad, 2000) and red wine (Waterhouse, 2002), are a family of flavonoid polyphenols associated with chemopreventive effects on colon, skin, lung, prostate, and breast cancers (Butt and Sultan, 2009). They are also associated with numerous cardiovascular benefits, such as decreased inflammation and platelet adhesion, increased endothelial nitric oxide activity, and improved blood lipid profile (Babu and Liu, 2008). Although the addition of catechins and other polyphenols can increase the functionality of a product, they can also elicit significant levels of bitterness and astringency (Peleg et al., 1999) - attributes generally associated with lower consumer acceptance (Lesschaeve and Noble, 2005) - which may reduce the update of these products in the market and place limits on the concentration of polyphenolic compounds that can be used in their formulation. These considerations have led to renewed interest in strategies to moderate the perception of bitterness and astringency elicited by these and related functional ingredients.

While a range of approaches have traditionally been used for decreasing bitterness in food and beverages, not all are desirable for use in functional food systems. For instance, the addition of sucrose and sodium chloride is less than optimal for these products as it may be perceived to lower their healthiness (Gaudette and Pickering, 2011a), and thus conflict with the core 'purpose' and positioning of functional foods.

Bitter blocking compounds offer an alternative approach, and a recent study investigated their efficacy at reducing the bitterness of (+)-catechin and caffeine in model solutions (Gaudette and Pickering, 2011b). Of the five compounds examined, β-cyclodextrin and homoeriodictyol sodium salt were the most effective at decreasing the bitterness of (+)-catechin, while the bitterness of caffeine was reduced most effectively by homoeriodictyol sodium salt. However, appreciable bitterness remained in all formulations, consistent with other studies.

Additional, and potentially complementary, methods include the use of alternate tastants and odourants. For instance, it is well documented that sweeteners can decrease bitterness (Kroeze and Bartoshuk, 1985). While this is especially true for sucrose (Calviño et al., 1990), the relationship is less clear for non-nutritive, plant-based sweeteners such as rebaudioside A that may be particularly attractive in functional foods. In addition, some aroma compounds have been shown to suppress the intensity of some tastes, including sweetness and sourness (Gillan et al., 1983; Stevenson et al., 1999). Few studies have explored the role that odourants may have on modifying the perception of bitterness. Coffee and chocolate aromas increase the bitterness of caffeine in fat free milk (Keast, 2008), and added cocoa flavouring significantly increases the bitterness of cocoa beverages (Labbe et al., 2006). The addition of vanilla flavouring, congruent with sweet taste, can enhance bitterness in an unfamiliar bitter milk beverage (Labbe et al., 2006). Further investigation, especially pertaining to odours that may suppress bitterness perception, is warranted.

At some concentrations, basic taste eliciting compounds, including sucrose, quinine and caffeine, can suppress the astringency elicited by phenolics (Brennan et al.,

2001). Also, fruity aromas can decrease the astringency of some white wine (Sáenz-Navajas et al., 2010). While molecular binding between polyphenols and aroma compounds has been shown to occur in model solutions (Dufour and Bayonove, 1999), the relative impact of this on the perception of astringency is unclear, and in some instances, may be minor. In addition, it is unclear how the concurrent addition of tastants may mediate the interaction between odourants and astringency perception. Thus, further examination into the influence of aroma and taste in modifying both the astringency and bitterness elicited by phenolic compounds is timely and of interest. (+)-Catechin represents a useful 'model' molecule for such a study, as it is relatively inexpensive, is currently used in functional food/beverage formulations (Gaudette and Pickering, 2011a), and elicits both bitterness and astringency at relatively low concentrations (Peleg et al., 1999; Gaudette and Pickering, 2011b).

The objective of this study, therefore, is to determine the sensory impact of select bitter blockers, sweeteners and odourants on the bitterness and astringency of (+)-catechin in aqueous solutions. The efficacy of these three flavour-modifying elements will be examined individually and in all combinations; we hypothesize that the bitterness and/or astringency elicited by the (+)-catechin solutions will decrease as more flavour-modifiers are added to the system. We also hypothesize that the impact of odourants will be mediated by their degree of cognitive association with bitterness and astringency. Specifically, we expect that odours congruent with sweetness will decrease bitterness/astringency, whereas odours congruent with bitterness or astringency will enhance their perception, consistent with some findings in other modalities and systems (Delwiche, 2004). Finally, we hypothesize that sweeteners that elicit a bitter side taste

will be less effective at decreasing the bitterness of (+)-catechin solutions than sweeteners that do not.

MATERIAL AND METHODS

Selection of stimuli

Stimuli and concentrations used for data collection are shown in Table 5.1. The concentration of (+)-catechin hydrate (CAT; Sigma-Aldrich, Oakville, Ontario, Canada) was based on our previous study (Gaudette and Pickering, 2011b). ß-cyclodextrin (CYCLO; Sigma-Aldrich) and homoeriodictyol sodium salt (HED; Symrise AG, Holzminden, Germany) were selected as the bitter blockers as they were the best performing compounds in the previous study, and because they operate via different mechanisms; molecular encapsulation for CYCLO (Szjetli, 1988) and purported interaction with the extracellular portion of TAS2Rs for HED (Ley et al., 2005). Sucrose (Sigma-Aldrich) and rebaudioside A (REB; PureCircle USA, Inc.) were selected as sweeteners, representing a traditional and plant-based, non-caloric sweetening compound, respectively. Concentrations were based on Schiffman et al. (1995) and tested for isosweetness through bench testing (n = 7, data not shown) (Table 1). Vanillin (VAN; Sigma-Aldrich) and black tea aroma (TEA; Firmenich, Inc., New Jersey, USA) were chosen to represent aromas cognitively associated with sweetness and bitterness/astringency, respectively. The concentration of black tea aroma was initially based on the manufacturer's recommendations, and adjusted after bench testing to be isointense with vanillin.

Table 5.1. Stimuli administered during data collection.

Control	CAT
Binary solutions	CAT + sweetener, bitter blocker or odourant
Ternary solutions	CAT + sweetener + bitter blocker
	CAT + sweetener + odourant
	CAT + bitter blocker + odourant
Quaternary solutions	CAT + sweetener + bitter blocker + odourant

CAT: (+)-catechin (6.2×10^{-3} M); sweetener: rebaudioside A (2.6×10^{-4} M) or sucrose (1.3×10^{-1} M); bitter blocker: ß-cyclodextrin [0.30 % (w/v)] or homoeriodictyol sodium salt (3.1×10^{-4} M); odourant: vanillin (6.6×10^{-4} M) or black tea extract (1.0 mL/L).

Participants, screening and training

Participants were staff or students of Brock University's Cool Climate Oenology and Viticulture Institute (CCOVI) (n=15, 34 ± 10 years, 7 males) between the ages of 22 and 52. All volunteered their time and signed consent forms that had been cleared by Brock University's Research Ethics Board. All training and data collection sessions were held in CCOVI's dedicated sensory evaluation lab.

Screening and training of participants involved correctly identifying and rating the intensity of eight aqueous solutions, each containing a taste (sweet, sour, salty, bitter, umami), astringent or in-mouth aroma (tea, vanilla) stimulus. Compounds and

concentrations were based on previous literature (Keast, 2003; Green and George, 2004; Pickering et al., 2006), modified as needed through bench testing; sucrose (sweetness, 3.0 x 10^{-1} M), citric acid (sourness, 3.0 x 10^{-3} M), sodium chloride (saltiness, 1.5 x 10^{-1} M), L-glutamic acid monosodium salt hydrate (umami, 1.0 x 10^{-1} M), quinine monohydrochloride dihydrate (bitterness, 5.0 x 10^{-5} M), aluminum sulfate (astringency, 4.4 x 10^{-4} M), vanillin (vanilla aroma, 6.6 x 10^{-4} M), and black tea aroma (1.0 mL/L).

In order to familiarize participants with scale usage, intensity ratings were recorded on a 15 cm visual analog scale with 1 cm indented anchors (bottom anchor of 'absent', top anchor of 'high'). Participants were first presented a single flight of solutions in International Organization for Standardization (ISO) wine tasting glasses capped with plastic lids. Each glass contained 20 mL of a single stimulus, and was labeled with the appropriate sensation. Instructions for tasting and rating were the following: *remove the lid from the glass, take the full amount of solution into the mouth, swirl for 5 seconds, expectorate the sample, wait at least 5 seconds for sensation to fully develop, rate the highest intensity experienced for the sensation on the line scale, rinse thoroughly 4 times with water, wait a minimum of 1 minute between samples.* After a mandatory 10-minute break, a second flight of the same solutions was randomly presented in glasses labeled with 3-digit, randomized codes and capped with plastic lids. The same instructions for tasting and rating were followed, and in addition, participants were asked to correctly identify the sensation.

Screening and training were considered completed when participants correctly identified and rated each stimulus presented in the second flight. An error in correctly identifying any sensation resulted in the participant being invited back for an additional

training and screening session. If an error in identification occurred during this additional session, participant was thanked and excused from participating in the remainder of the study (n=0).

Data collection

Samples and preparation

Treatments administered during data collection sessions consisted of aqueous solutions of (+)-catechin alone (control), or as part of binary, ternary, or quaternary mixtures with sweeteners, bitter-blocking, and/or odourants (Table 5.1). All solutions were prepared using fresh deionized water (Millipore RiOs 16 Reverse Osmosis System, MA, USA). 1 mL of 99% pure food grade ethanol (Liquor Control Board of Ontario, St. Catharines, Ontario, Canada) was used to fully solubilize (+)-catechin prior to its addition to solutions made in 100 mL volumetric flasks. This concentration of ethanol is below perception threshold in water (Thorngate and Noble, 1995). Samples were prepared in foil-wrapped volumetric flasks to protect (+)-catechin from possible light-induced polymerization (Peleg et al., 1999). Samples were then transferred to airtight, 120 mL amber coloured glass bottles (Fisher Scientific, Rockford, IL, USA). The headspace was filled with nitrogen gas, and samples stored in darkness at 4°C and replaced every 5 days. Based on our previous work (Gaudette and Pickering, 2011b), (+)-catechin is stable and remains in monomeric form during these storage conditions for at least 5 days.

Design and data analysis

A restricted randomized block design was used. Replication was blocked, with initial and duplicate assessments presented in randomized order. All possible combinations of (+)-catechin together with each sweetener, bitter blocker and odourant were assessed as binary, ternary or quaternary solutions. Thus, 26 different solutions and one control [(+)-catechin] were presented to all participants, in duplicate. These 54 samples were presented over 9 separate sessions, with each session consisting of two flights of three samples. Forced breaks of 3 and 10 minutes, along with 4 water rinses, were enforced between samples and flights, respectively. Samples were removed from the refrigerator 1 hour prior to testing. 20 mL of each sample were then poured into ISO wine glasses and labeled with randomized 3-digit numbers.

Participants' responses were collected using a computerized program (Compusense c5v4, 111 Farquhar St., Guelph, Ontario, Canada N1H 3N4). Five 15 cm line scales with 1 cm indented anchors (bottom anchor 'absent', top anchor 'high') were presented for each sample on a single screen, and each sample was rated for intensity of bitterness, astringency, sweetness, in-mouth aroma, and 'other' (a term to capture any additional sensations perceived). All evaluations took place under red lighting to mask any visual differences between the samples. For each sample, the following tasting protocol/instructions was strictly adhered to: *remove lid from glass, take the full solution into the mouth, swirl in mouth for 5 seconds, expectorate the sample, wait 10 seconds for sensations to fully develop* (participants were verbally instructed to wait longer if intensity was still building after 10 seconds), *rate the highest intensity experienced for each sensation, rinse thoroughly with water at least 4 times during the forced 3 minute*

break, continue onto the next sample only when mouth is completely free from all taste, tactile, and in-mouth aroma sensations.

Data treatment and statistical analyses

Statistical analysis was performed using XLSTAT version 2011.1.01 for Apple Macintosh (Addinsoft, USA). Initial analyses were performed to determine individual participant performance and to identify possible outliers. Three criteria were used to assess participant performance for the bitterness responses: (1) *Reproducibility between replicates*. Coefficient of variation between each duplicate measurement, averaged across all treatments, of < 100%. (2) *Bitterness sensitivity*. Intensity ratings for (+)-catechin > 1.0 cm averaged across both replicates. (3) *Discrimination*. p(F) > 0.05 for treatment from a 2-way ANOVA that included bitterness ratings from all treatments and control as the dependent variable (rep and treatment as independent variables). If a participant failed two or more of these criteria, they were removed from the dataset (n=2).

Separate two-way ANOVAs were then conducted for the (+)-catechin + binary, (+)-catechin + ternary and (+)-catechin + quaternary solutions. The dependent variables for each of these analyses were the intensity ratings of the five sensations. For each participant, the averaged ratings across both replicates were used. For each initial ANOVA, treatment, participant and their interaction were included as the independent variables. The ANOVA was repeated with the interaction term removed if it was not significant (p(F)>0.05). Tukey's HSD mean separation tests were used for post-hoc analyses ($\alpha = 0.05$). To assist in the interpretation of figures, displayed intensity ratings,

captured on a 15 cm visual analog scale, were converted to a score out of 100.

RESULTS AND DISCUSSION

Bitterness of (+)-catechin in binary solutions

2-way ANOVA results demonstrate a main effect for bitterness from treatment [($F(6,72) = 3.9$, ($p<0.01$)] and judge [($F(12,72) = 19.6$, ($p<0.0001$)]. There was no significant treatment x judge interaction. A significant reduction in the bitterness of (+)-catechin is seen with (+)-catechin + sucrose (Figure 5.1). Sucrose and REB reduced the bitterness of the (+)-catechin solution by 41% and 37%, respectively, although the effect of REB was not significant. Alternate sweeteners, including REB, have previously been reported to elicit bitterness (Schiffman et al., 1995); thus the slightly reduced efficacy of REB compared with sucrose may be due to a bitter side-taste. No other binary solution treatments significantly decreased the bitterness of (+)-catechin.

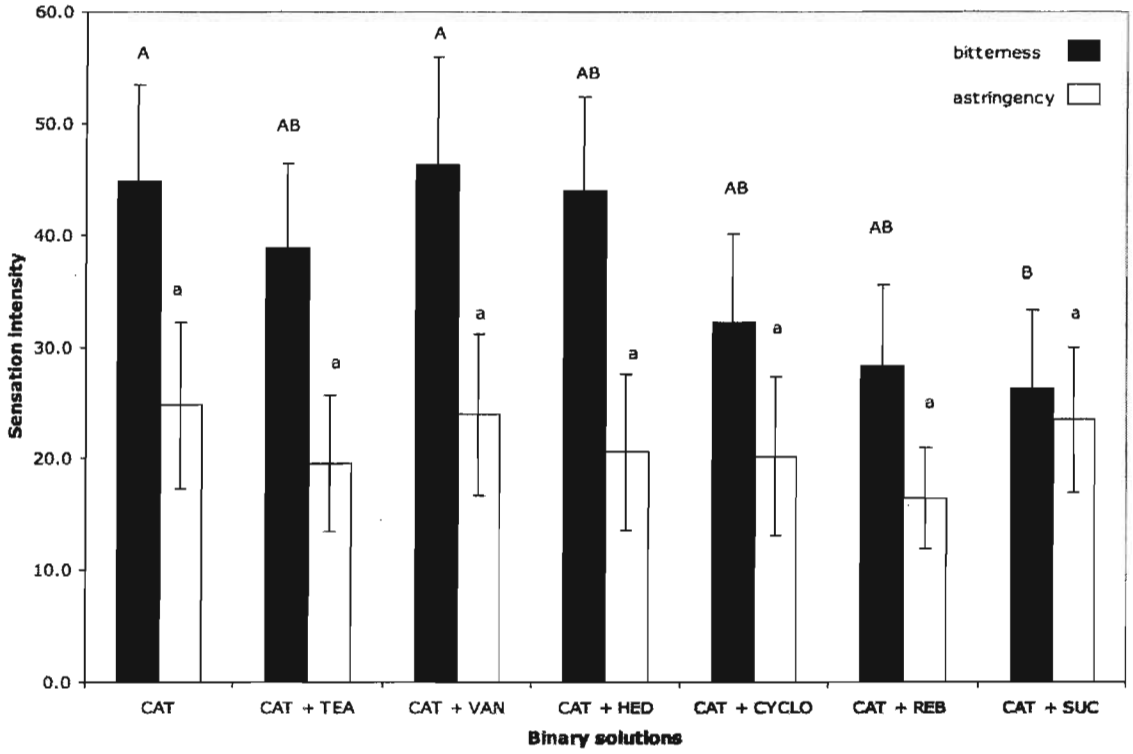

Figure 5.1. Perceived bitterness and astringency of (+)-catechin in binary solutions containing sweeteners, bitter blockers or odourants. Values represent mean responses from duplicate measurements ± SEM (n=13). Means sharing the same letter do not differ significantly across treatments for bitterness (uppercase) or astringency (lowercase) (Tukey's $HSD_{0.05}$). CAT [(+)-catechin; 6.2 x 10^{-3} M]; SUC (sucrose; 1.3 x 10^{-1} M); REB (rebaudioside A; 2.6 x 10^{-4} M); VAN (vanillin; 6.6 x 10^{-4} M); TEA (black tea aroma; 1.0 mL/L); CYCLO (ß-cyclodextrin; 0.30 %); HED (homoeriodictyol sodium salt; 3.1 x 10^{-4} M).

Bitterness of (+)-catechin ternary solutions

2-way ANOVA results demonstrate a main effect for bitterness from treatment [($F(12,144) = 5.6$, ($p<0.0001$)] and judge [($F(12,144) = 26.7$, ($p<0.0001$)]. There was no significant treatment x judge interaction ($p>0.05$). The bitterness of (+)-catechin is significantly decreased in ternary solutions containing sweeteners + bitter blockers (Figure 2). Additions of CYCLO + sweeteners are particularly effective, with CYCLO + SUC and CYCLO + REB decreasing bitterness by 60% and 68%, respectively. The bitterness of (+)-catechin is not significantly altered by TEA + REB or vanillin + REB, however, it is significantly decreased when either odourant is combined with sucrose. As postulated for the binary solutions, this difference may be attributed to bitterness elicited by REB.

Sweeteners + odourants and sweeteners alone (Figure 5.2) elicit very similar bitterness responses, indicating that the addition of odourants does not confer additional bitter reducing effects. Also, bitterness reduction observed for bitter blockers is not enhanced by the addition of odourants to the mixtures, providing further evidence of the ineffectiveness of these odourants in modifying the bitterness elicited by (+)-catechin.

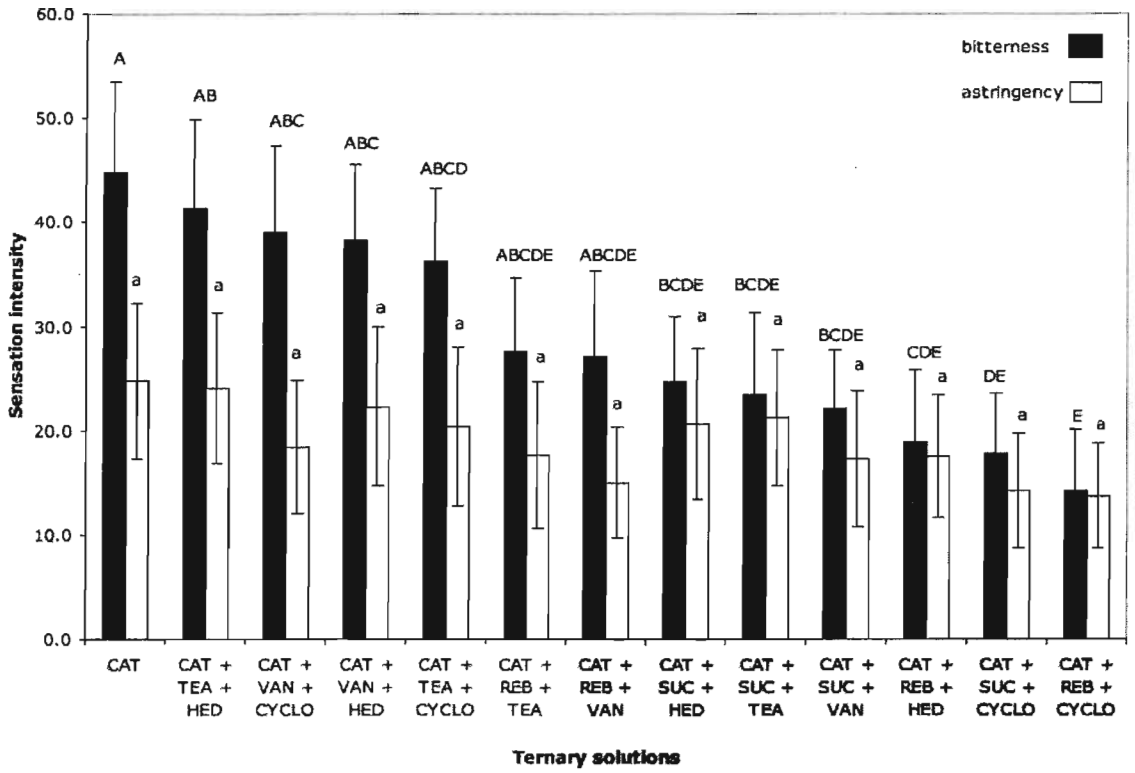

Figure 5.2. Perceived bitterness and astringency of (+)-catechin in ternary solutions containing sweeteners, bitter blockers and/or odourants. Values represent mean responses from duplicate measurements ± SEM (n=13). Means sharing the same letter do not differ significantly across treatments for bitterness (uppercase) or astringency (lowercase) (Tukey's $HSD_{0.05}$). CAT [(+)-catechin; 6.2 x 10^{-3} M]; SUC (sucrose; 1.3 x 10^{-1} M); REB (rebaudioside A; 2.6 x 10^{-4} M); VAN (vanillin; 6.6 x 10^{-4} M); TEA (black tea aroma; 1.0 mL/L); CYCLO (ß-cyclodextrin; 0.30 %); HED (homoeriodictyol sodium salt; 3.1 x 10^{-4} M).

Bitterness of (+)-catechin in quaternary solutions

2-way ANOVA results demonstrate a main effect for bitterness from treatment [($F(8,96) = 7.9$, ($p<0.0001$)] and judge [($F(12,96) = 20.8$, ($p<0.0001$)]. There was no

significant treatment x judge interaction ($p>0.05$). The bitterness of (+)-catechin is significantly decreased by quaternary solutions containing sweeteners + bitter blockers + odourants (Figure 3). This is particularly evident for solutions containing CYCLO. An overall trend is evident of lower bitterness responses for CYCLO- versus HED-containing solutions. On average, HED- and CYCLO- containing quaternary solutions decrease the bitterness of (+)-catechin by 46% and 65%, respectively. This difference is further supported by a 2-sample t-test showing that the average bitterness of CYCLO-containing solutions is significantly lower that HED- containing solutions [$t(102) = 2.1$; $p<0.05$].

Post-hoc Tukey's HSD results indicate no significant difference in bitterness between quaternary solutions differing only by odourant type. This is in agreement with the results from the binary and ternary solutions. Despite the cognitive association with sweetness and bitterness that exists for vanilla and black tea odours, respectively, their addition in these solutions does not significantly alter the bitterness of (+)-catechin.

Astringency and other sensations

The astringency intensity elicited by the (+)-catechin only solutions was approximately 54% of the bitterness responses. 2-way ANOVA results show no effect of binary solutions on the astringency of (+)-catechin. For ternary and quaternary solutions, main effects for treatment [($F(12,144) = 2.1$, ($p<0.05$); ($F(8,96) = 2.9$, ($p<0.01$), respectively] and judge [($F(12, 144) = 81.9$, ($p<0.0001$); ($F(12,96) = 75.4$, ($p<0.0001$), respectively] are observed. There was no significant treatment x judge interactions for any treatment ($p>0.05$).

Post-hoc Tukey's HSD failed to separate the treatment means. However, CYCLO + sweeteners trend toward being the most effective ternary solutions in decreasing astringency (Figure 5.2). In quaternary solutions, half of the CYCLO- containing treatments significantly decrease astringency, with an average reduction of 44% (Figure 5.3). These mixtures are also the most effective at lowering bitterness. The responses for CYCLO- containing quaternary treatments do not, however, differ from each other. Odourants in binary solutions do not significantly affect astringency (Figure 5.1), consistent with the results from ternary (Figure 5.2) and quaternary (Figure 5.3) mixtures.

2-way ANOVA results demonstrate a main effect for sweetness and in-mouth aroma, respectively, from treatment in binary [($F(6,1) = 91287$, ($p<0.01$); ($F(6,1) = 5221$, ($p<0.05$)], ternary [($F(12,144) = 27.2$, ($p<0.0001$); ($F(12,144) = 5.0$, ($p<0.0001$)] and quaternary solutions [($F(8,96) = 11.0$, ($p<0.0001$); ($F(8,96) = 8.5$, ($p<0.0001$)]. A main effect was observed for sweetness and in-mouth aroma, respectively, from judge in binary [($F(12,1) = 5158$, ($p<0.05$); ($F(6,1) = 2786$, ($p<0.05$)], ternary [($F(12,144) = 7.5$, ($p<0.0001$); ($F(12,144) = 10.4$, $p<0.0001$)] and quaternary [($F(8,96) = 10.6$, ($p<0.0001$); ($F(8,96) = 17.6$, ($p<0.0001$)] solutions. A judge x treatment interaction was found in binary solutions for sweetness and in-mouth aroma, respectively [($F(72,1) = 468$, ($p<0.05$); ($F(6,1) = 3686$, ($p<0.05$)].

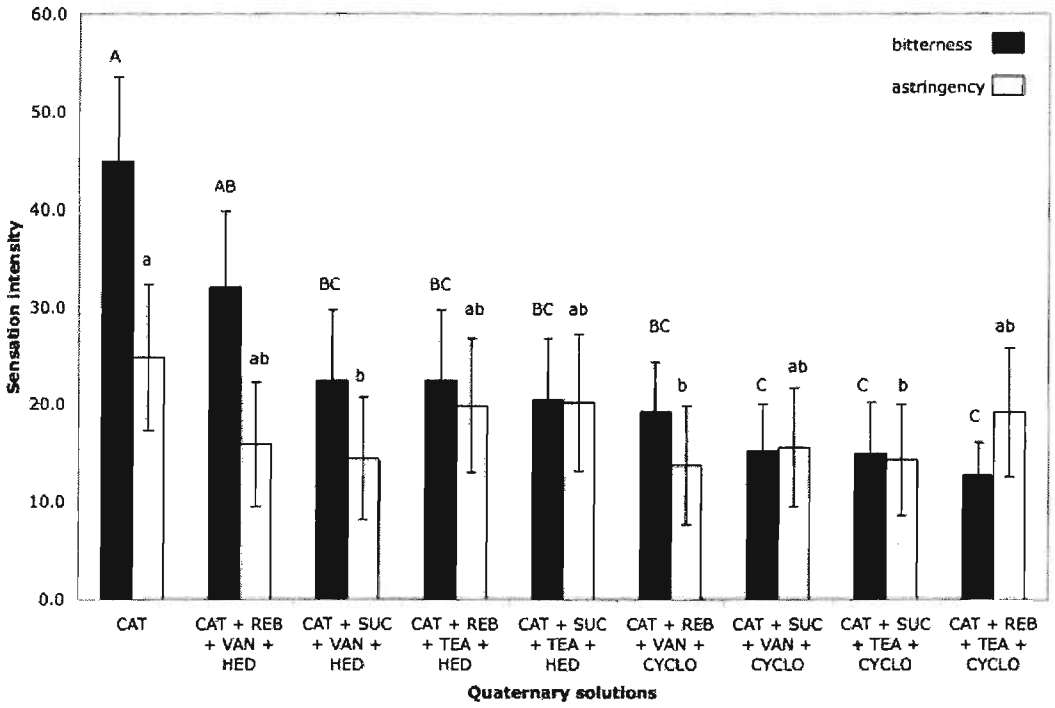

Figure 5.3. Perceived bitterness and astringency of (+)-catechin in quaternary solutions containing sweeteners, bitter blockers and odourants. Values represent mean responses from duplicate measurements ± SEM (n=13). Means sharing the same letter do not differ significantly across treatments for bitterness (uppercase) or astringency (lowercase) (Tukey's $HSD_{0.05}$). CAT [(+)-catechin; 6.2 x 10^{-3} M]; SUC (sucrose; 1.3 x 10^{-1} M); REB (rebaudioside A; 2.6 x 10^{-4} M); VAN (vanillin; 6.6 x 10^{-4} M); TEA (black tea aroma; 1.0 mL/L); CYCLO (ß-cyclodextrin; 0.30 %); HED (homoeriodictyol sodium salt; 3.1 x 10^{-4} M).

As expected, Tukey's HSD results show that solutions containing sweeteners and odours were rated significantly higher in sweetness and in-mouth aroma, respectively, compared to solutions that did not contain these compounds (data not shown). 2-way ANOVA results did not show a main treatment effect for 'other' sensations in binary,

ternary, or quaternary solutions.

Other considerations

We hypothesized that the degree of bitterness and/or astringency inhibition would increase with the number of flavour modifying compounds added to an aqueous solution of (+)-catechin. Our results demonstrate that the combination of sweeteners + ß-cyclodextrin in ternary solutions is most effective at decreasing the bitterness and astringency of (+)-catechin. This reduction in bitterness and astringency can be attributed to both a central cognitive mechanism, from sweetness, and a physiological effect from ß-cyclodextrin encapsulating (+)-catechin, consistent with the reduction in bitterness observed with zinc sulfate and Na-cyclamate in Keast and Breslin (2005). Of interest, increasing the number of flavour modifiers from 2 to 3 (ternary solutions and quaternary solutions, respectively) does not confer an additional reduction in perceived bitterness or astringency.

Due to the bitterness that can be elicited by REB (Schiffman et al., 1995), we hypothesized that it would be less effective compared to sucrose in decreasing the bitterness of (+)-catechin. Our results show that REB and sucrose generally confer similar levels of bitterness inhibition in binary, ternary and quaternary solutions. Therefore, we conclude that REB may be an increasingly valuable ingredient in the development of low and non-sugar foods and beverages, including various functional foods and diabetic products.

We also hypothesized that the addition of odours cognitively associated with sweetness and bitterness/astringency would suppress or enhance the bitterness and/or astringency of (+)-catechin, respectively. However, the odourants did not alter bitterness nor astringency ratings in binary solutions, and furthermore, does not provide additional reducing or enhancing effects of these sensations in ternary and quaternary solutions. We suggest further testing of odour-bitterness interactions using a wider range of odourants and concentrations.

It is important to acknowledge that model aqueous solutions were used in this study, and therefore some caution should be exercised in applying these approaches and results to real food and beverage formulations, given that they are complex matrices that involve numerous interrelated components. For instance, interactions between the physical, chemical and rheological elements of real food/beverages may mediate the efficacy of the flavour modifying elements and combinations examined here. Nonetheless, this study provides valuable preliminary data that may guide industry formulations of some functional products, and we recommend expanding these results to include further testing on more complex matrices.

Finally, we acknowledge that gross variation exists among individuals in the ability to perceive bitterness and astringency. While we did not quantify this possible variation in the present study, it may be of value to determine whether the efficacy of bitter blockers, sweeteners, and odourants on bitterness and astringency varies with individual variation in orosensory perception. In particular, markedly different orosensory responsiveness reflected in 6-*n*-propylthiouracil (PROP) (Bartoshuk et al., 1994) and thermal (Cruz and Green, 2000; Bajec and Pickering, 2008) tasting phenotypes

may anticipate that some populations of individuals will respond differently to the flavour modifiers and combinations examined here.

CONCLUSION

Sweeteners used in combination with ß-cyclodextrin effectively reduce the bitterness and astringency of (+)-catechin, and may have application for functional food formulations. Rebaudioside A is equally as effective as sucrose, which may be promising for product developers creating low sugar or diabetic products. The addition of odourants congruent with sweetness or bitterness does not alter the bitterness or astringency of (+)-catechin. Future work could incorporate and apply these results to a wider array of health-promoting, plant-based bitterants in order to better determine the best approach for flavour optimization.

Chapter 6 - THE CONTRIBUTION OF BITTER BLOCKERS AND SENSORY INTERACTIONS TOWARDS FLAVOUR PERCEPTION

Nicole J. Gaudette, Jeannine F. Delwiche, and Gary J. Pickering

The candidate is the primary author and contributor to this chapter. As co-author, Dr. Jeannine Delwiche contributed insight and direction with respect to statistical analysis. Dr. Gary Pickering has provided various edits throughout the development of this chapter.

This work is to be submitted to the journal *Flavour*.

INTRODUCTION

Flavour is arguably the most important driver in determining the consumer acceptance and purchasing behaviour for functional food products (Siró et al., 2008). However, flavour can be compromised in some of these formulations due to the addition of certain bioactive ingredients. For example, at higher concentrations, the addition of plant-derived compounds can elicit less than optimal levels of bitterness and astringency (Gomez-Carneros and Drewnowski, 2000). This in turn, may lead to a decrease in the consumer acceptance of these products (Lesschaeve and Noble, 2005). Thus, a major challenge facing industry is to create products that contain an adequate level of ingredients to fulfill bioactivity requirements, yet have a sensory profile that is acceptable to consumers.

Flavour perception is a complex cognitive phenomenon that involves the combination of various orosensory sensations, including taste, smell and mouthfeel. Numerous interactions can exist between these sensations, and thus, changes to one within a mixture may affect another and modify the overall perceived flavour (Delwiche, 2004). Within a food matrix, interactions can occur within the same (taste-taste) or across (taste-aroma, taste-mouthfeel) sensory modalities. Overall, interactions within the same sensory modality are more effective at altering flavour compared to those across modalities (Gillan, 1983).

Sensory interactions within a food or beverage matrix result from a central cognitive or an oral physiological mechanism. A central cognitive effect can occur between tastants, where the intensity of one tastant in a mixture is perceived as

suppressed or enhanced independent of physical interactions that occur between tastants within the oral cavity (Keast and Breslin, 2002). For example, through split-tongue studies, the addition of sucrose (sweet tasting) to a quinine solution (bitter tasting) results in the mutual suppression of sweetness and bitterness (Kroeze and Bartoshuk, 1985). A central cognitive effect can also occur between tastes and odours, where the perceived sweetness of sucrose is enhanced by the addition of a congruent odour, such as strawberry (Frank and Byram, 1988). However, taste-odour pairings that are typically not associated with each other do not result in taste enhancement (e.g., sweet taste and peanut butter odour) (Frank and Byram, 1988). Odour-induced enhancement of taste is based on prior associations made through food and beverage behaviour (Delwiche, 2004), and thus, occur due to perceptual processes (Small and Prescott, 2005).

Sensory interactions may also be based on an oral physiological effect, whereby the perceived intensity of a stimulus is dependent on physiological interactions with other stimuli in the mouth (Keast and Breslin, 2002). The suppression of bitterness by saltiness is an example of this effect (Breslin and Beauchamp, 1995, 1997). Salt-induced suppression of bitterness may be due to a number of reasons, including alteration of the integrity of the TAS2R receptor cell wall or interaction with the TAS2R taste transduction pathway (Keast and Breslin, 2002).

While numerous sensory interactions have been studied in conventional foods and beverages, investigations using functional foods and beverages are needed. Formulations for these products are continually evolving, and can include the addition of bitter modifying compounds, or 'bitter blockers.' Bitter blockers lower the perception of

bitterness by targeting a bitterant through molecular encapsulation, creating a physical barrier to decrease bitterant-receptor binding, or by interaction with the extracellular polypeptide chain of TAS2Rs (reviewed in Gaudette and Pickering, 2011a). However, the addition of bitter blockers into a mixture with various orosensory stimuli may alter their bitter inhibiting capacity, and/or impact the interactions that occur between these stimuli, and thus, affect the overall perceived flavour. The influence that these compounds may have on the sensory interactions and thus, the overall flavour profile of a functional food product, has yet to be understood.

There are two main objectives of this study. The first is to investigate the impact of within (taste-taste) and cross-modal (taste-odour) sensory interactions on the overall flavour profile of (+)-catechin-containing aqueous solutions. However, in many food formulations, additional flavour modifying compounds such as bitter blockers may be used. Therefore, the second objective is to determine the impact of bitter blockers on the overall flavour profile of (+)-catechin-containing aqueous solutions. (+)-Catechin represents a useful 'model' molecule for such a study, as it is relatively inexpensive, is currently used in functional food/beverage formulations (Gaudette and Pickering, 2011a), and elicits both bitterness and astringency at relatively low concentrations (Peleg et al., 1999; Gaudette and Pickering, 2011b).

We hypothesize that within modality, taste-taste (bitter-sweet) interactions will be more effective than cross-modality, taste-odour (bitter-vanillin) interactions at decreasing the bitterness and astringency of (+)-catechin. In binary taste-odour matrices, we expect sweet-associated odours to enhance and suppress sweetness and bitterness, respectively.

For bitter associated odours, we expect the opposite effect. In ternary matrices, two different tastants will be present (bitter and sweet) in addition to an odourant. We anticipate that vanilla, an aroma cognitively associated with sweetness, will result in the enhancement of sweetness, and as a result, decrease the perceived bitterness of (+)-catechin. We hypothesize that black tea, an aroma cognitively associated with bitterness, will lead to an odour-induced enhancement and suppression of bitterness and sweetness, respectively.

We further hypothesize that the addition of bitter blockers to aqueous solutions of (+)-catechin containing sweeteners or odourous compounds will alter the perceived sweetness and aroma intensity of these solutions. Addition of bitter blockers that target TAS2Rs to a mixture could decrease the bitterness of (+)-catechin, and as a result, may also increase the intensity of sweetness and aroma. However, some bitter blockers decrease bitterness via molecular encapsulation. Thus, additional stimuli (sweeteners, odours) within a mixture also may become bound, resulting in a decrease in their perceived intensity. Information obtained here will serve as a basis for the application of these flavour modifiers within the functional food industry.

MATERIAL AND METHODS

As done in Chapter 5.

Data treatment and statistical analyses

Statistical analysis was performed using XLSTAT version 2011.1.01 for Apple Macintosh (Addinsoft, New York, NY, USA). Initial analyses were performed to determine individual participant performance and to identify possible outliers. Three criteria were used to assess participant performance for the bitterness responses: (1) *Reproducibility between replicates*. Coefficient of variation between each duplicate measurement, averaged across all treatments, of < 100%. (2) *Bitterness sensitivity*. Intensity ratings for (+)-catechin > 1.0 cm averaged across both replicates. (3) *Discrimination*. $p(F) < 0.05$ for treatment from a 2-way ANOVA that included bitterness ratings from all treatments and control as the dependent variable (rep and treatment as independent variables). If a participant failed two or more of these criteria, they were removed from the dataset (n=2).

Separate two-way ANOVAs were conducted to examine the following interactions on the perception of (+)-catechin in aqueous solutions: *taste +odour* [binary and ternary solutions containing REB, SUC, V, and T alone, and in all possible taste-odour combinations], *taste + bitter blocker* [binary and ternary solutions containing REB, SUC, HD, and CD alone, and in all possible taste-bitter blocker combinations], *bitter blocker +odour* [binary and ternary solutions containing HD, CD, T and V alone, and in all possible bitter blocker-odour combinations]. The dependent variables for each of these analyses were the intensity ratings of bitterness, astringency, sweetness, or in-mouth aroma. For each participant, the averaged ratings across both replicates were used. For each initial ANOVA, treatment (solutions corresponding to *taste + odour*, *taste + bitter blocker*, or *bitter-blocker + odour* groupings), participant and their interaction were included as the independent variables. The ANOVA was repeated with the interaction

term removed if it was not significant ($p(F)>0.05$). Tukey's HSD mean separation tests were used for post-hoc analyses ($\alpha = 0.05$). To assist in the interpretation of figures, displayed intensity ratings, captured on a 15 cm visual analog scale, were converted to a score out of 100.

An agglomerative hierarchical cluster analysis (Ward's method) was performed on all treatments, including control, to determine groups with a similar flavour profile. Variables for this analysis were the intensity ratings of bitterness, astringency, sweetness, and in-mouth aroma, with ratings for each variable averaged across replicate. Intensity ratings for 'other' were not included in the analysis. A minimal level of additional sensations was perceived for these treatments (average rating for intensity across all treatments = 7.4 out of 100, and no significant difference in 2-way ANOVA for participant and treatment as independent variables and intensity rating for 'other' as dependent variable [$F(26,312) = 1.47$, ($p>0.05$)]), and thus, 'other' was not included in the analysis.

To determine the relative effect sizes of within- and cross-modal sensory interactions on the intensity of various sensations, Cohen's D calculations were performed. Cohen's D value = $(M_1 - M_2)/\sigma$ pooled (Cooper and Hedges, 1994). Effect sizes are interpreted as small if D = 0.2, medium if D = 0.5, large if D = 0.8 (Cohen, 1988).

RESULTS

Taste-odour interactions

For the impact of taste-odour interactions on the in-mouth aroma intensity of vanilla and black tea, 2-way ANOVA results demonstrate a main effect for treatment [($F(8,1) = 4336.3$, ($p<0.05$)], judge [($F(12,1) = 5103.5$, ($p<0.05$)], and treatment x judge [($F(95,1) = 454.8$, ($p<0.05$)]. For the impact of taste-odour interactions on the sweetness elicited by sucrose and rebaudioside A, 2-way ANOVA results demonstrate a main effect for treatment [($F(8,1) = 114913.6$, ($p<0.01$)], judge [($F(12,1) = 27596.5$, ($p<0.01$)], and treatment x judge [($F(95,1) = 5715.5$, ($p<0.05$)]. For the impact of taste-aroma interactions on the bitterness elicited by (+)-catechin, 2-way ANOVA results demonstrate a main effect for treatment [($F(8,96) = 4.6$, ($p<0.0001$)] and judge [($F(12,96) = 21.3$, ($p<0.0001$)]. No treatment x judge resulted from this analysis ($p>0.05$).

Results reveal the impact of taste-odour interactions on the perception of bitterness, sweetness, and in-mouth aromas of (+)-catechin-containing aqueous solutions (Figure 6.1). Overall, the aroma intensity of vanilla combined with (+)-catechin is significantly increased by the addition of sucrose and rebaudioside A. Sweetener addition, however, does not alter the perceived intensity of black tea aroma.

The sweetness intensity of sucrose combined with (+)-catechin is significantly increased by the addition of vanillin and black tea odourants. However, an increase in sweetness intensity does not occur in solutions containing rebaudioside A. Interestingly, the odour-induced enhancement of sweetness elicited by sucrose does not result in a

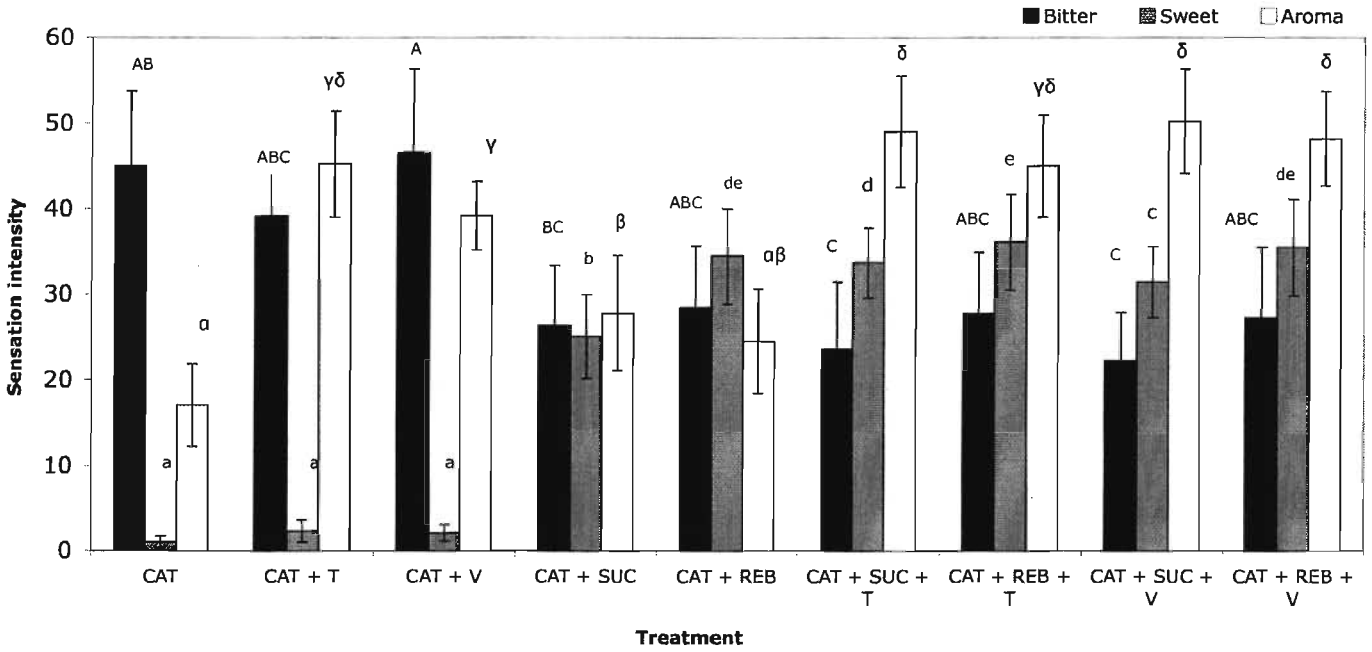

Figure 6.1. Perceived bitterness, sweetness and in-mouth aroma elicited by (+)-catechin aqueous solutions containing sweeteners and/or odourants. Values represent mean responses from duplicate measurements ± SEM (n=13). Means sharing the same letter do not differ significantly across treatments for bitterness (uppercase), sweetness (lowercase), and in-mouth aroma (Greek letters) (Tukey's $HSD_{0.05}$). CAT [(+)-catechin; 6.2×10^{-3} M]; SUC (sucrose; 1.3×10^{-1} M); REB (rebaudioside A; 2.6×10^{-4} M); V (vanillin; 6.6×10^{-4} M); T (black tea aroma; 1.0 mL/L).

significant decrease in bitterness compared to sucrose alone. Also, the bitterness intensity of (+)-catechin is not significantly affected by the addition of vanillin or black tea odourants.

Compared to (+)-catechin alone, a trend of lower bitterness is observed in both binary and ternary solutions with added sweeteners. On average, all binary and ternary treatments (with bitter blockers or odourants) with added sweeteners result in a 48% decrease in bitterness. The bitterness suppressing effect of sweetness in these solutions is further supported through hierarchical cluster analysis, where all sweetener-containing

treatments are grouped separately from those without sweeteners (Figure 6.2). Furthermore, within these sweetener-containing treatments, additional groupings result between those containing bitter blockers or odourants. Overall, conditions clustered together suggest a similarity in the overall perceived flavour profile.

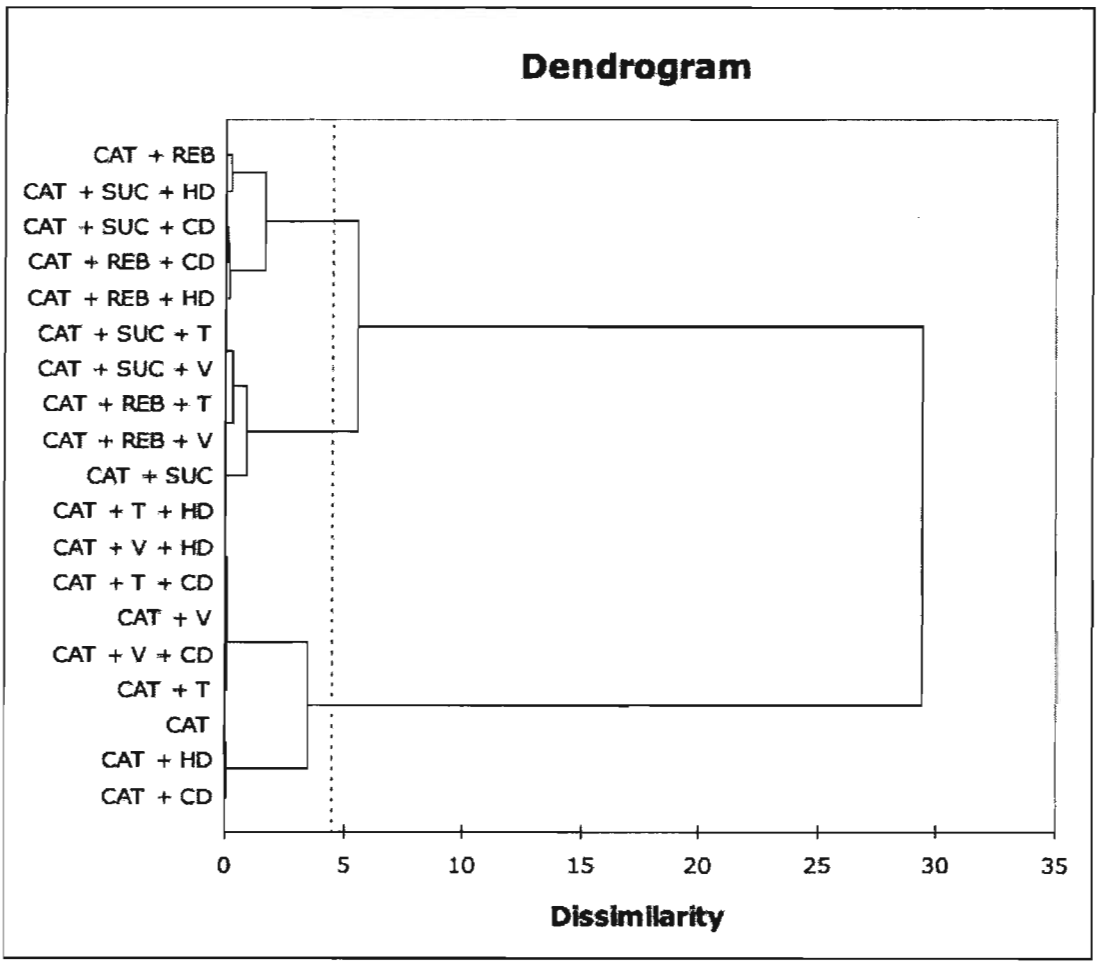

Figure 6.2. Agglomerative hierarchical cluster analysis dendrogram that segments all binary and ternary conditions into groups of similar perceived flavour profile. Dotted line denotes level of dissimilarity along which clusters are determined. CAT [(+)-catechin; 6.2 x 10^{-3} M]; SUC (sucrose; 1.3 x 10^{-1} M); REB (rebaudioside A; 2.6 x 10^{-4} M); V (vanillin; 6.6 x 10^{-4} M); T (black tea aroma; 1.0 mL/L); CD (ß-cyclodextrin; 0.30 %); HD (homoeriodictyol sodium salt; 3.1 x 10^{-4} M).

Taste-bitter blocker interactions

For the impact of taste-bitter blocker interactions on the astringency intensity of (+)-catechin, 2-way ANOVA results demonstrate a main effect for treatment [($F(8,96) = 4.3$, ($p<0.05$)] and judge [($F(12,96) = 92.1$, ($p<0.0001$)]. For the impact of taste-bitter blocker interactions on the sweetness intensity of sucrose and rebaudioside A, 2-way ANOVA results demonstrate a main effect for treatment [($F(8,96) = 24.0$, ($p<0.0001$)] and judge [($F(12,96) = 4.2$, ($p<0.0001$)]. For the impact of taste-bitter blocker interactions on the bitterness intensity of (+)-catechin, 2-way ANOVA results demonstrate a main effect for treatment [($F(8,96) = 7.5$, ($p<0.0001$)] and judge [($F(12,96) = 21.0$, ($p<0.0001$)]. No treatment x judge interaction resulted from these analyses ($p>0.05$).

Results show a significant increase in sweetness of (+)-catechin + rebaudioside A combined with ß-cyclodextrin compared to (+)-catechin + rebaudioside A and homoeriodictyol sodium salt. However, neither combination is perceived significantly sweeter compared to (+)-catechin + rebaudioside A alone (Figure 6.3).

The astringency of (+)-catechin is significantly decreased by the addition of rebaudioside A + ß-cyclodextrin, although, this does not result in a significantly lower perceived astringency compared to rebaudioside A alone. No other treatments were effective at modifying the astringency of (+)-catechin.

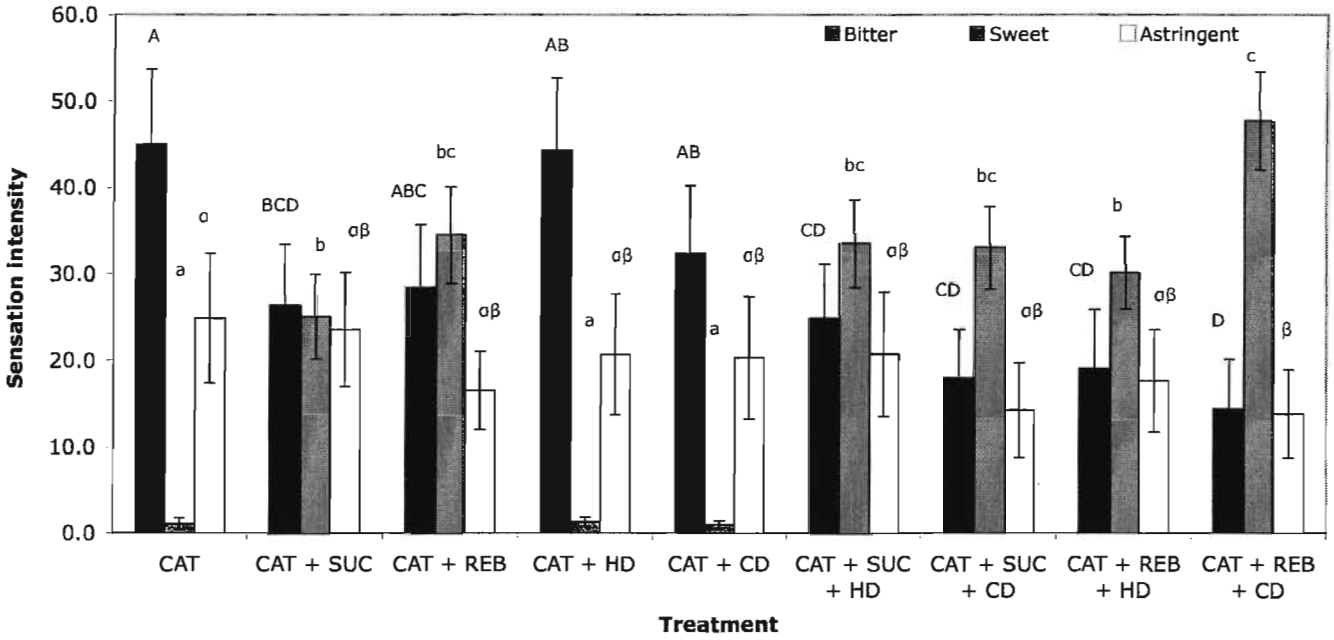

Figure 6.3. Perceived bitterness, sweetness and astringency of (+)-catechin aqueous solutions containing sweeteners and/or bitter blocking compounds. Values represent mean responses from duplicate measurements ± SEM (n=13). Means sharing the same letter do not differ significantly across treatments for bitterness (uppercase), sweetness (lowercase), and astringency (Greek letters) (Tukey's $HSD_{0.05}$). CAT [(+)-catechin; 6.2 x 10^{-3} M]; SUC (sucrose; 1.3 x 10^{-1} M); REB (rebaudioside A; 2.6 x 10^{-4} M); CD (ß-cyclodextrin; 0.30 %); HD (homoeriodictyol sodium salt; 3.1 x 10^{-4} M).

All combinations of bitter blockers + sweeteners decrease the bitterness of (+)-catechin, but this is not significantly more effective compared to sweeteners alone.

Odour-bitter blocker interactions

For the impact of aroma-bitter blocker interactions on the in-mouth aroma intensity of vanilla and black tea, 2-way ANOVA results demonstrate a main effect for treatment [($F(8,96) = 9.8$, ($p<0.0001$)] and judge [($F(12,96) = 5.5$, ($p<0.0001$)]. No

treatment x judge interaction resulted from this analysis ($p>0.05$). Aroma-bitter blocker interactions did not affect the bitterness or astringency of (+)-catechin ($p>0.05$).

Binary and ternary treatments containing odourants are perceived significantly higher in aroma intensity compared to treatments without odourants. No differences result between ternary solutions containing bitter blockers + odourants, and binary solutions containing odourants alone. The addition of bitter blockers and odourants to (+)-catechin aqueous solutions does not significantly modify the bitterness and astringency of (+)-catechin (data not shown).

Effect size of within-modal versus cross-modal sensory interactions

The effect size of various within-modal and cross-modal sensory interactions on the perceived intensity of orosensory sensations is presented in Table 6.2.

Within-modal

Sweeteners had a larger inhibiting effect on the bitterness compared to the astringency elicited by (+)-catechin.

Cross-modal

Sweeteners have a larger effect on the perceived aroma intensity of vanilla compared to black tea. Sweet-odour interactions have a larger effect on decreasing the bitterness compared to the astringency elicited by (+)-catechin. Odours have a larger effect on the perceived sweetness of sucrose compared to rebaudioside A.

Overall effect on bitterness and astringency: Cross-modal versus within-modal

Within-modal sensory interactions have a stronger effect on decreasing bitterness and astringency compared to cross-modal (Table 6.2). On average, the effect size of

odours on bitterness is -0.09, while the effect size of sweeteners is -0.60. A similar impact results for astringency, where the average effect sizes of odours and sweeteners are -0.13 and -0.28, respectively.

Table 6.1 Effect sizes of cross-modal and within-modal sensory interactions on the perception of orosensory sensations. Size is determined using the Cohen's D* value calculation. Effect sizes are interpreted as small if D = 0.2, medium if D = 0.5, large if D = 0.8

CROSS-MODAL INTERACTION	OVERALL EFFECT MEASURED	SENSATION RATED	COHEN'S D VALUE
Sweetness-induced effect	(CAT + REB + V) - (CAT + V)	Vanilla aroma	0.53
		Bitterness	-0.40
		Astringency	-0.38
	(CAT + SUC + V) - (CAT + V)	Vanilla aroma	0.59
		Bitterness	-0.67
		Astringency	-0.27
	(CAT + REB + T) - (CAT + T)	Black tea aroma	-0.30
		Bitterness	-0.40
		Astringency	-0.08
	(CAT + SUC + T) - (CAT + T)	Black tea aroma	0.15
		Bitterness	-0.50
		Astringency	0.09
Odour-induced effect	(CAT + V + REB) - (CAT + REB)	Sweetness	0.07
		Bitterness	-0.02
		Astringency	-0.08
	(CAT + T + REB) - (CAT + REB)	Sweetness	0.10
		Bitterness	-0.03
		Astringency	0.03
	(CAT + V + SUC) - (CAT + SUC)	Sweetness	0.36
		Bitterness	-0.18
		Astringency	-0.26
	(CAT + T + SUC) - (CAT + SUC)	Sweetness	0.53
		Bitterness	-0.10
		Astringency	-0.09
Odour-induced effect on bitterness	(CAT + V) - CAT	Bitterness	0.04
	(CAT + T) - CAT	Bitterness	-0.21
Odour-induced effect on astringency	(CAT + V) - CAT	Astringency	-0.03
	(CAT + T) - CAT	Astringency	-0.22
WITHIN-MODAL INTERACTION			
Sweetness-induced effect on bitterness	(REB + CAT) - CAT	Bitterness	-0.57
	(SUC + CAT) - CAT	Bitterness	-0.64
Sweetness-induced effect on astringency	(REB + CAT) - CAT	Astringency	-0.36
	(SUC + CAT) - CAT	Astringency	-0.20

*Cooper and Hedges, 1994.

DISCUSSION

We hypothesized that within (taste-taste) versus cross-modality (taste-odour) orosensory interactions would be more effective at decreasing the bitterness and astringency of (+)-catechin. Overall, our results show that the application of sweeteners is more effective than odourants at decreasing the bitterness and astringency of (+)-catechin.

We also anticipated that sweet-associated odours would suppress bitterness and enhance sweetness. Odourants had no impact on the perception of bitterness. However, vanilla increased the perception of sweetness, although this did not lead to decreased bitterness as we predicted. The basis for such cognitive mechanisms is primarily due to prior taste-odour associations. Thus, it is possible that vanilla-induced enhancement of sweetness did not lead to a decreased perception of bitterness due to the novelty of the model beverage. A similar result was found by Labbe et al. (2006), where a bitter milk beverage was not perceived as less bitter when a vanilla odour was added. In addition, if bitterness suppression were to result in these solutions from the odour-induced enhancement of sweetness, it would involve both within and cross-modality sensory interactions. Since cross-modal interactions are less able to affect perception compared to within-modal interactions (Gillan, 1983), sweetness enhancement by vanilla may not confer a strong enough cognitive effect to alter the perception of bitterness.

Sucrose-induced enhancement of vanilla aroma is a newly discovered phenomenon that has been reported by Green et al. (2011). We demonstrated that both sucrose and rebaudioside A enhanced the intensity of vanilla aroma, and thus, provide further evidence of this cross-modal enhancement of in-mouth aroma. In addition,

rebaudioside A - a plant-derived, alternative sweetener - confers a similar level of aroma enhancement compared to sucrose, which may be of value in certain sweet-tasting food formulations. Sweet taste-induced enhancement of black tea aroma did not occur, most likely due to the lack of prior associations between these sensations.

Astringency was significantly decreased by ß-cyclodextrin + rebaudioside A. Here, it is possible that encapsulation of (+)-catechin by ß-cyclodextrin decreased the interaction of (+)-catechin and proline-rich salivary proteins in the oral cavity. Further reduction of astringency may have resulted through cognitive suppression from the sweetness elicited by rebaudioside A. Thus, both oral peripheral and central cognitive effects may have resulted in a decrease in the perceived astringency of (+)-catechin.

Similarly, the application of bitter blockers and sweet-eliciting compounds results in a significant decrease in the bitterness of (+)-catechin. Here, both oral physiological and central cognitive effects impact the taste profile of (+)-catechin in aqueous solutions. While it is known that sucrose decreases bitterness via a central cognitive effect (Kroeze and Bartoshuk, 1985), it is unclear whether rebaudioside A is effective through the same mode of action, and thus, further investigation is warranted. The use of bitter blockers decreases bitterness via an oral physiological mechanism, with ß-cyclodextrin and homoeriodictyol sodium salt imparting their effects by molecular encapsulation (Szjetli, 1988) and possible interference with the extracellular portion of TAS2Rs (Ley et al., 2005), respectively.

Finally, the application of bitter blockers did not alter the perceived aroma intensity of odourants. In addition, the perception of sweeteners was not altered. Thus,

we conclude that the perception of these odourants and sweeteners is not altered when used in matrices containing ß-cyclodextrin and homoeriodictyol sodium salt.

CONCLUSION

Orosensory interactions involving taste and smell are an integral part of the perception and modification of flavour. In functional food formulations, the application of bitter blockers are an additional flavour modifying strategy that may contribute to and/or impact the various orosensory interactions present in a matrix. Overall, the addition of ß-cyclodextrin and homoeriodictyol sodium salt to solutions containing sweeteners and odourants does not effect the perception of these stimuli. In contrast, sweeteners in particular, and vanilla aroma impact the flavour profile of (+)-catechin solutions by suppressing bitterness and enhancing sweetness, respectively.

The results reported here are limited to the compounds, concentrations and matrices used in this study. However, future formulations could involve the use of these flavour-modifying strategies towards improving the overall flavour profile of functional food and beverages. Thus, further investigation into the impact that these variables may have in real functional food and beverage matrices containing other bitter and astringency eliciting functional ingredients is warranted.

Chapter 7 - GENERAL DISCUSSION AND CONCLUSION

Summary and Discussion

The functional food and beverages sector is an important area of current and future growth for the food industry. These products are fortified with various health-promoting ingredients, and offer an attractive alternative to conventional foods for health-conscious consumers.

There is an increasing interest in the development of functional products fortified with plant-derived ingredients. Plant-based functional ingredients are perceived as more natural and healthy compared to animal-based and synthetic compounds, and thus, are becoming increasingly valuable as fortifying agents (West et al., 2002). In recent years, research has become increasingly focused on the bioactivity of grape- and green tea-derived polyphenolic compounds. These ingredients demonstrate protection against various diseases and thus, may be particularly valuable additions to a food or beverage system.

It is well known that flavour is an important driver of food choice and consumer acceptance. While fortification with plant-derived bioactive compounds, such as polyphenols, may become increasingly valuable, at certain concentrations they can elicit higher levels of bitterness and astringency - sensations that are not typically acceptable to consumers when perceived at high levels (Lesschaeve and Noble, 2005). As the number and diversity of products introduced into the functional food and beverage market increases, new flavour modifying strategies may be needed to create products with an

acceptable sensory profile. Thus, a considerable challenge for the functional food and beverage industry exists: to create products that contain levels of fortifying agents sufficient enough for effective bioactivity, but that elicit a flavour profile that is acceptable to consumers.

The first aim of this investigation was to explore the flavour of functional beverages and model solutions fortified with plant-based, health-relevant bitterants. Here, the sensory properties of *trans*-resveratrol, a bioactive ingredient in the functional food and nutraceutical industry, were determined in a functional wine product. Overall, fortification with *trans*-resveratrol does not impact the sensory profile of Cabernet Sauvignon. Fortification of Riesling at 200 mg/L elicits higher levels of bitterness compared to 20 mg/L and control (0 mg/L). Furthermore, fortified wines have a higher antioxidant capacity compared to control, *trans*-resveratrol remains in solution over a 58-week shelf life period, and changes to the chemical profile of fortified wines are minimal (Chapter 3). While fortification with *trans*-resveratrol may be successful for some wine styles, further research on the enrichement of other wine styles is warranted.

In some functional food and beverage formulations, flavour modification is needed in order to improve the flavour profile of a product. While bitterness modification is a major focus for the food industry, traditional strategies, such as the addition of sucrose and sodium chloride, do not support the 'healthiness' associated with functional food products. Alternative methods to traditional flavour modifying strategies are needed to improve the flavour profile of some functional food products. A critical review of current approaches and alternative methods was presented in this dissertation (Chapter 2). Overall, bitter blocking compounds may offer an attractive alternative

flavour modification strategy for the functional food industry. However, it is relatively unclear how effective these compounds may be towards decreasing the bitterness of plant-derived, health-relevant bitterants that are currently used in functional food and beverage formulations.

The second aim of this investigation was to examine the effect that alternative flavour modifying strategies, such as bitter blocking compounds, may have on the bitterness intensities of caffeine and (+)-catechin (Chapter 4). Here, high and low concentrations of five different blockers were tested for their efficacy in decreasing the bitterness of high and low concentrations of caffeine and (+)-catechin - 2 health-promoting, plant-derived compounds that are used as fortifying agents in the functional food industry.

It was hypothesized that the efficacy of bitter blockers would be dependent upon bitterant and bitter blocker mode of action. Overall, application of homoeridictyol sodium salt and ß-cyclodextrin were most effective at decreasing the bitterness of (+)-catechin, while only homoeridictyol sodium salt significantly decreased the bitterness of caffeine. Thus, bitter inhibiting capacity of bitter blockers is bitterant dependent, and may be based on differing bitter blocker modes of action.

While bitter blockers may offer an alternative approach to flavour modification, the use of alternative sweeteners and odourants may provide an additional bitter reducing effect in certain food and beverage matrices. It is well known that sweetness can suppress bitterness, and the impact of odourants on tastes can lead to their suppression or enhancement (Delwiche, 2004). However, the overall impact that bitter blockers,

sweeteners, and odourants may have on the bitterness intensity and overall flavour profile of plant-derived, health-promoting bitterants is unknown.

The third aim of this investigation was to determine the overall effect of bitter blockers, alternative, plant-based sweeteners and odourants on the flavour profile of (+)-catechin in aqueous solutions (Chapters 5 and 6). It was hypothesized that an increasing number of flavour modifiers added to a solution of (+)-catechin would lead to a stronger bitter reducing effect. However, this was not fully supported, as quaternary solutions did not provide additional bitter inhibiting effects compared to ternary solutions. Ternary solutions containing sucrose or rebaudioside A - a plant-based alternative sweetener - paired with ß-cyclodextrin were most effective at decreasing the bitterness of (+)-catechin. Thus, in certain matrices, fewer flavour modifiers may be more effective towards decreasing the bitterness perceived by certain functional ingredients.

In addition, odourants did not alter the bitterness or astringency of (+)-catechin (Chapter 5). While the application of odourous compounds examined here did not impact the bitterness or astringency elicited by (+)-catechin, additional work using a larger range of odourants to further explore this relationship is warranted.

It was also hypothesized that within- compared to cross-modal sensory interactions would have a stronger bitter and astringent inhibiting effect on (+)-catechin in aqueous solutions. This prediction was supported here, as binary and ternary solutions containing sweet-bitter interactions were more effective compared to vanilla-bitter. In addition, a vanilla-induced enhancement of sweetness intensity, and a sweet-induced enhancement of vanilla aroma resulted (Chapter 6). This supports previous reports for the existance of vanilla-induced enhancement of sweetness (Green and George, 2004),

and sweetness-induced enhancement of vanilla (Green et al., 2011). However, the enhancements of sweetness and vanilla did not reduce the bitterness elicited by (+)-catechin. With regards to product formulation, additional investigation involving the impact of taste on aroma intensity could be of particular value. Taste-induced aroma enhancement could be an important strategy that would permit the use of lower concentrations of certain odourants, and ultimately lead to a more economical formulation process for industry.

Flavour perception of matrices containing both taste and aroma-eliciting ingredients can be difficult to predict, as various and complex sensory interactions are involved in the perception of the overall flavour profile. However, the impact of cross-modal sensory interactions on flavour is known to confer a lesser effect compared to within-modal interactions. Thus, the overall flavour of complex matrices may be more likely to be impacted by within- versus cross-modal sensory interactions.

Conclusion

While flavour modification is widely used in the food industry, traditional methods are often sub-optimal for use in functional food and beverage formulations. Overall, alternative strategies, including the use of bitter blocking compounds and non-caloric plant-based sweeteners, may be useful in decreasing the bitterness elicited by certain plant-derived, health-promoting ingredients.

It is important to acknowledge that a limited number and concentration of bitterants, bitter blockers and other flavour modifiers were tested in this investigation. Thus, recommendations for the use of these modifiers in formulations containing other

bitterants should be made with caution. In addition, due to the impact of individual variation in orosensory perception, it may not be accurate to relay the results presented here towards certain populations due to their taste sensitivity. Thus, additional work involving these populations may be of value.

Overall, this work has provided a basis for the future implementation of alternative flavour modifiying strategies aimed specifically towards the formulation of functional beverages. As the functional food industry continues to grow and evolve, the applications investigated here may assist in future formulation development, as developers continue to strive towards the goal of creating bioactive products with an acceptable flavour profile.

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APPENDIX A: *TRANS*-RESVERATROL STANDARD CURVE (CHAPTER 3)

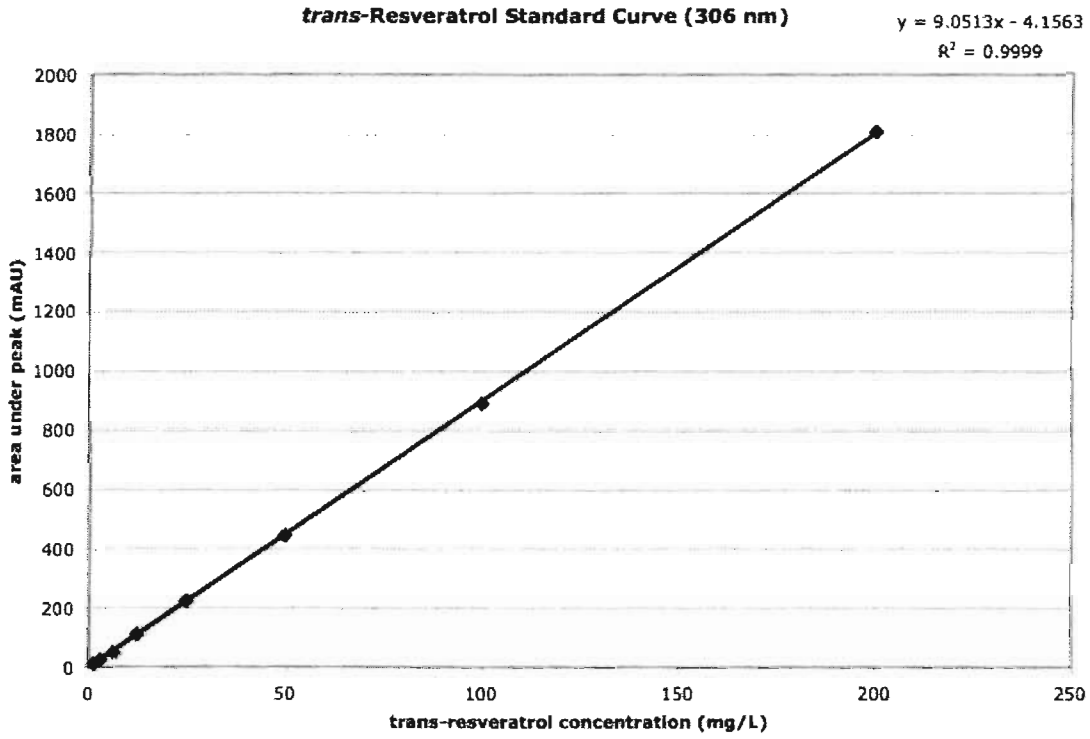

Figure A1. *trans*-Resveratrol standard curve. Data represent mean values of duplicate standards.

APPENDIX B: *CIS*-RESVERATROL STANDARD CURVE (CHAPTER 3)

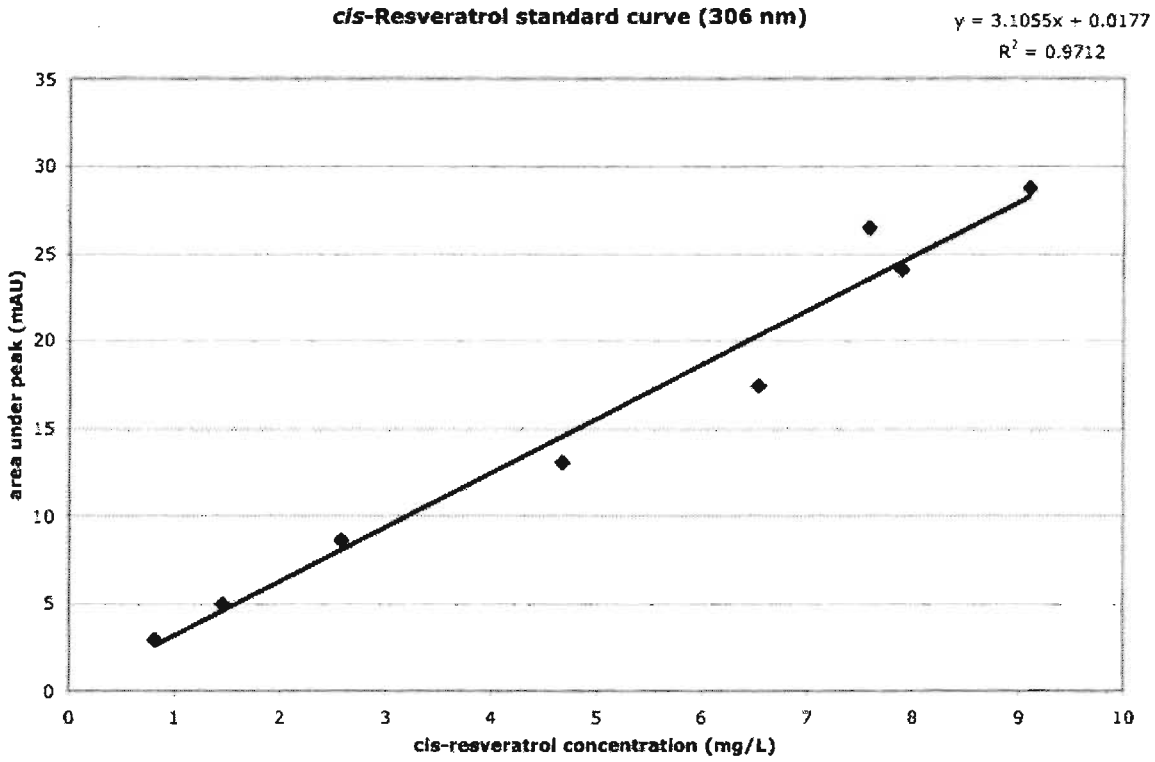

Figure A2. *cis*-Resveratrol standard curve. Data represent mean values of duplicate standards.

APPENDIX C: TROLOX EQUIVALENTS ANTIOXIDANT CAPACITY (TEAC) STANDARD CURVE (CHAPTER 3)

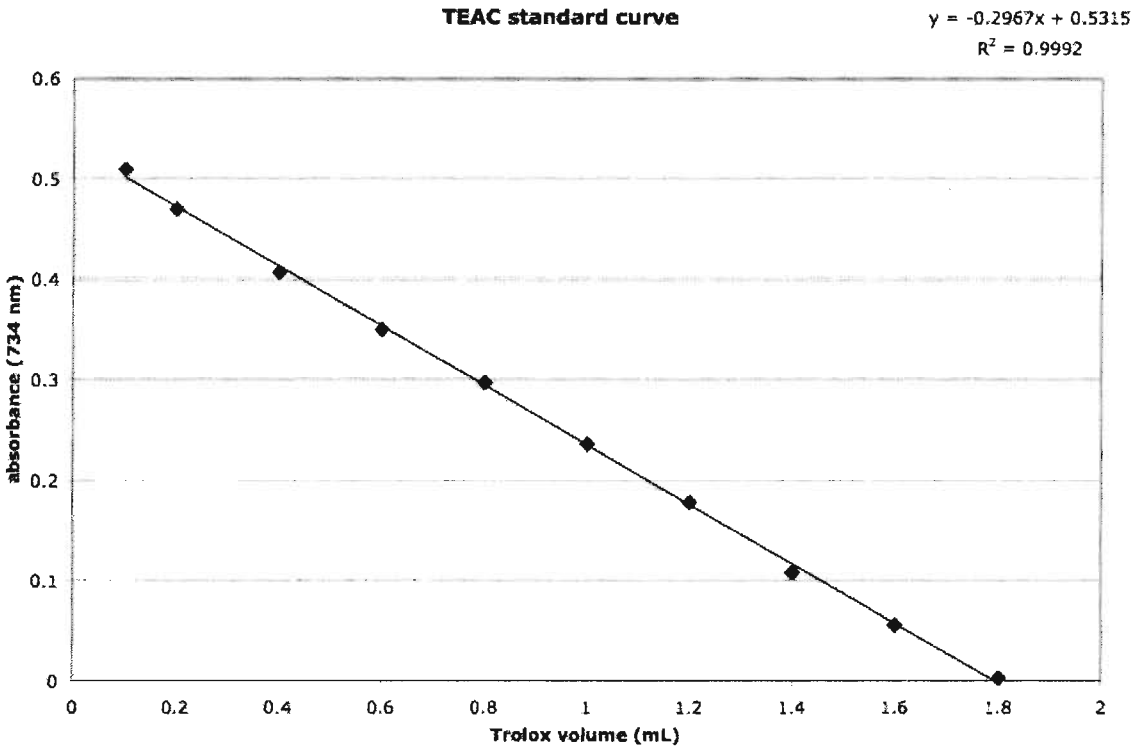

Figure A3. Trolox equivalents antioxidant capacity (TEAC) standard curve. Data represent mean values of duplicate standards.

APPENDIX D: pH, TA AND FREE SO_2 VALUES FOR CABERNET SAUVIGNON AND RIESLING WINES (CHAPTER 3).

Table A1. pH, TA and free SO_2 values for Cabernet Sauvignon and Riesling wines. Data represent mean values of duplicate measurements from duplicate bottles ± standard deviations. Means sharing the same letter do not differ significantly across time [lowercase] or at a specific time point [uppercase] (Fisher's $LSD_{0.05}$). TA = titratable acidity

Cabernet Sauvignon	**Bottling**	**6 weeks**	**18 weeks**	**31 weeks**	**44 weeks**	**58 weeks**
pH						
0 mg/L	3.11 ± 0.00 Aab	3.14 ± 0.01 Aa	3.10 ± 0.03 Aab	3.14 ± 002 Aab	3.14 ± 0.01 Aab	3.01 ± 0.02 Ab
20 mg/L	3.12 ± 0.00 Aa	3.13 ± 0.01 Aa	3.07 ± 0.00 Ab	3.11 ± 0.00 Aa	3.14 ± 0.02 Aa	3.06 ± 0.00 Ab
200 mg/L	3.16 ± 0.00 Aab	3.19 ± 0.09 Aa	3.13 ± 0.02 Aab	3.11 ± 0.02 Aab	3.16 ± 0.01 Aab	3.08 ± 0.01 Ab
TA						
0 mg/L	6.56 ± 0.05 Aa	6.70 ± 0.07 Ab	6.75 ± 0.05 Ab	6.62 ± 0.04 Ac	6.09 ± 0.04 Ad	6.06 ± 0.20 Ad
20 mg/L	6.23 ± 0.00 Ba	6.32 ± 0.04 Ba	6.74 ± 0.16 Ab	6.49 ± 0.10 Bc	6.28 ± 0.04 Ba	5.98 ± 0.17 Ad
200 mg/L	6.53 ± 0.11 Aa	6.36 ± 0.15 Bb	6.57 ± 0.13 Bc	6.56 ± 0.10 ABc	6.21 ± 0.04 Cd	5.49 ± 0.46 Be
Free SO_2						
0 mg/L	18.4 ± 1.13 Aa	13.2 ± 2.01 Ab	10.4 ± 1.27 Ac	8.60 ± 0.77 Ac	8.80 ± 0.92 Ac	8.40 ± 1.53 Ac
20 mg/L	17.6 ± 0.00 Aa	14.4 ± 2.26 Aa	10.1 ± 1.20 Ab	7.00 ± 1.20 Bc	10.0 ± 2.01 Ab	6.40 ± 0.00 Bc
200 mg/L	17.6 ± 0.00 Aa	13.6 ± 0.92 Ab	10.8 ± 0.90 Ac	8.20 ± 0.40 ABd	7.60 ± 0.80 Ade	6.80 ± 0.80 Be
Riesling	**Bottling**	**6 weeks**	**18 weeks**	**31 weeks**	**44 weeks**	**58 weeks**
pH						
0 mg/L	3.09 ± 0.00 Aa	3.12 ± 0.01 Aa	3.15 ± 0.01 Aa	3.13 ± 0.02 Aa	3.16 ± 0.01 Aa	3.07 ± 0.02 Aa
20 mg/L	3.07 ± 0.00 Aab	3.11 ± 0.01 Abc	3.08 ± 0.01 Bab	3.16 ± 0.02 Ad	3.15 ± 0.01 Acd	3.07 ± 0.00 Aa
200 mg/L	3.08 ± 0.00 Aa	3.08 ± 0.02 Aa	3.09 ± 0.01 Ba	3.18 ± 0.03 Ab	3.15 ± 0.02 Ab	3.06 ± 0.00 Aa
TA						
0 mg/L	7.50 ± 0.00 Aab	7.45 ± 0.07 Aa	7.55 ± 0.03 Ac	7.37 ± 0.04 Ab	6.71 ± 0.08 Ad	7.07 ± 0.15 Ae
20 mg/L	6.79 ± 0.05 Ba	7.45 ± 0.11 Ab	7.72 ± 0.04 Bc	7.46 ± 0.18 Bb	6.88 ± 0.04 Bc	6.88 ± 0.04 Bd
200 mg/L	7.39 ± 0.05 Aa	7.33 ± 0.04 Ab	7.77 ± 0.03 Bc	7.46 ± 0.04 Bd	6.99 ± 0.07 Ba	6.58 ± 0.50 Ce
Free SO_2						
0 mg/L	13.6 ± 1.13 Aa	10.4 ± 0.92 Ab	7.48 ± 0.82 Ac	5.60 ± 2.07 Ad	4.28 ± 1.51 Ad	4.40 ± 0.80 Ad
20 mg/L	14.4 ± 0.00 Aa	8.40 ± 1.53 Ab	7.20 ± 0.75 Ab	7.20 ± 0.92 Ab	3.88 ± 0.70 Ac	4.40 ± 0.80 Ac
200 mg/L	15.2 ± 1.13 Aa	8.80 ± 0.65 Ab	7.28 ± 0.59 Ac	6.40 ± 1.31 Ac	4.20 ± 1.51 Ad	6.00 ± 0.80 Bc

APPENDIX E: CONSENT FORMS (CHAPTER 3)

INFORMATION STATEMENT AND CONSENT FORM

Name of Research Project: Determination of olfactory, taste, and visual threshold of *trans*-resveratrol enriched wine.

Department/Institute: Brock University; Department of Biological Science/ Cool Climate Oenology and Viticulture Institute

Principle Investigator: Ms. Nicole Gaudette, MSc. Student, Dept. of Biological Sciences, Brock University, (905) 688-5550 ext: 4719, ng06qz@brocku.ca

Supervisor: Dr. Gary Pickering, Associate Professor & Chair, Dept. of Biological Sciences, Brock University, (905) 688-5550 ext: 4715, gpickeri@brocku.ca

The Project:

This study will determine the olfactory and taste threshold for *trans*-resveratrol in wine. *trans*-Resveratrol is a powerful antioxidant found naturally in wine. It is associated with a decrease in the proliferation of numerous disease states such as atherosclerosis and cancer, as well as an increase in the lifespan of microorganisms. Although many studies have shown this compound to have profound physiological effects, the population threshold of this important polyphenol are unknown. This information is key in order to create future wine products that are fortified with health-promoting polyphenols such as *trans*-resveratrol.

The Procedure:

You are invited to participate in this study! 25-30 participants will fill-out questionnaires about health, demographics, and wine involvement.

You will then be given 2 sets of 3 glasses (= 1 'set') containing wine. For each set you will be asked simply to identify which one is the 'odd' one (2 will be the same and 1 will be different).

This procedure will be repeated once more at a different session.

FOLLOWING ALL TASTINGS, SAMPLES MUST BE EXPECTORATED. FAILURE TO DO SO WILL REQUIRE YOU TO IMMEDIATELY WITHDRAW FROM THIS STUDY.

Benefits/Risks

The expected benefits: This research will contribute to determining the sensory thereshold of *trans*-resveratrol in wine. These results will help the industry to determine an acceptable level of *trans*-resveratrol fortification in commercial wines. You will also learn what your *trans*-resveratrol threshold is in wine, as well as gain an understanding of the methods used in psychophysical research.

The expected risks are no great than those encountered in normal daily food and beverage consumption.

Voluntary Participation:

You are free to withdraw your participation in the research at any time, and if you do, any data collected from will immediately be destroyed. You will not be subjected to any penalty or discriminatory treatment.

Responsibilities:

You need only schedule 4 blocks of time (15-20 min each time) to come to the CCOVI Sensory Lab at Brock University. Mutually convenient times will be negotiated with you.

Publication of Results

It is expected that the results of this study will be published, in academic journals and presented at conferences. Please feel free to contact Dr Pickering at any time should any questions arise, or for information on the progress or results of the study.

Confidentiality

All data will be confidential, individual identities will not be disclosed to anyone outside of the research team. Paper data collected in this study will be will be retained for 7 years. It will be stored in a locked, private area accessible only to Dr Pickering. An

electronic copy of the data will be retained indefinitely in case further analysis is warranted. Dr Pickering may use the data for recruiting purposes for further sensory research in this lab.

Ethics Clearance:

This study has been reviewed and received ethics clearance through the Brock (file # 07-017). If you have any comments or concerns about your rights as a research participant, please contact the Research Ethics Office at (905) 688-5550 Ext. 3035, reb@brocku.ca.

Consent:

The purpose of the research has been explained to me, including the potential risks/discomforts associated with the research. I have also been given the opportunity to ask questions about the research and received satisfactory answers, and know that I may continue to ask questions and receive satisfactory answers throughout the study.

I understand that I am free to withdraw my participation in the research at any time and that if I do I will not be subjected to any penalty or discriminatory treatment. I also understand my participation in this project is on a voluntary basis, and no remuneration will be provided by Brock University in exchange for my participation. I understand that I must expectorate wine samples following tasting, and that failure to comply with this will cause me to immediately withdraw from the study.

I understand that any information or personal details gathered in the course of this research about me are confidential and that neither my name nor any other identifying information will be used or published without my written permission.

The Brock University Research Ethics Board does not approve but clears this application for ethics review (file # 07-017).

I understand that if I have any complaints or concerns about this research I can contact: Research Ethics Officer, Office of Research Services, Brock University, Ph: 905 688 5550, ext: 3035; **reb@brocku.ca**

Your Name: .....

Signature:

.....

Date:.....

Please check this box if you ARE NOT interested in being contacted to participate in future sensory study performed by the Pickering lab. ☐

INFORMATION STATEMENT AND CONSENT FORM

Name of Research Project: Determining the flavour profile of *trans*-resveratrol enriched wine.

Department/Institute: Brock University; Department of Biological Science/ Cool Climate Oenology and Viticulture Institute

Principle Investigator: Ms. Nicole Gaudette, MSc. Student, Dept. of Biological Sciences, Brock University, (905) 688-5550 ext: 4719, ng06qz@brocku.ca

Supervisor: Dr. Gary Pickering, Associate Professor & Chair, Dept. of Biological Sciences, Brock University, (905) 688-5550 ext: 4715, gpickeri@brocku.ca

The Project:

This study will determine the flavour profile for *trans*-resveratrol in wine. *trans*-Resveratrol is a powerful antioxidant found naturally in wine. It is associated with a decrease in the proliferation of numerous disease states such as atherosclerosis and cancer, as well as an increase in the lifespan of microorganisms. Although many studies have shown this compound to have profound physiological effects, the population threshold of this important polyphenol are unknown. This information is key in order to create future wine products that are fortified with health-promoting polyphenols such as *trans*-resveratrol.

The Procedure:

You are invited to participate in this study! 12 participants will fill-out questionnaires about health, demographics, and wine involvement.

Stage 1: Formulation of aroma and taste descriptors

You will be given 3 white wines followed by 3 red wines. For each type of wine, 1 of the samples will be a control (no *trans*-resveratrol added), while the other 2 samples will contain increasing levels of *trans*-resveratrol. You will be asked to formulate both aroma and taste descriptors. As a group, we will then agree upon a number of descriptors for both the red and the white wines.

Stage 2: Rating of aroma and taste descriptor intensity

In a later session, you will individually rate the intensity of each descriptor (aroma followed by taste descriptors) using Compusense software located in the CCOVI sensory analysis room. A two minute mandatory break will occur between samples, as well as a 30 minute break occurring between sampling white and red wines.

FOLLOWING ALL TASTINGS, SAMPLES MUST BE EXPECTORATED. FAILURE TO DO SO WILL REQUIRE YOU TO IMMEDIATELY WITHDRAW FROM THIS STUDY.

Benefits/Risks:

The expected benefits: This research will contribute to determining the impact that *trans*-resveratrol has on the aroma and taste of wine. These results will help the industry to determine an acceptable level of *trans*-resveratrol fortification in commercial wines. . You will also learn what your *trans*-resveratrol threshold is in wine, as well as gain an understanding of the methods used in psychophysical research.

The expected risks are no great than those encountered in normal daily food and beverage consumption.

Voluntary Participation:

You are free to withdraw your participation in the research at any time, and if you do, any data collected from will immediately be destroyed. You will not be subjected to any penalty or discriminatory treatment.

Responsibilities:

You need only to schedule 4 blocks of time (45-60 min) to come to the CCOVI Sensory Lab at Brock University. Mutually convenient times between all panelists will be negotiated.

Publication of Results

It is expected that the results of this study will be published, in academic journals and presented at conferences. Please feel free to contact Dr Pickering at any time should any questions arise, or for information on the progress or results of the study.

Confidentiality

Due to the nature of descriptive analysis methodology, we cannot guarantee that the data collected will remain anonymous. As researchers, we cannot control the possibility of panelists disclosing information. Thus, we urge that you keep the discussion of this study confidential. Paper data collected in this study will be will be retained for 7 years. It will be stored in a locked, private area accessible only to Dr Pickering. An electronic copy of the data will be retained indefinitely in case further analysis is warranted. Dr Pickering may use the data for recruiting purposes for further sensory research in this lab.

Ethics Clearance:

The Brock University Research Ethics Board does not approve but clears this application for ethics review (file # 07-017). If you have any comments or concerns about your rights as a research participant, please contact the Research Ethics Office at (905) 688-5550 Ext. 3035, reb@brocku.ca.

Consent:

The purpose of the research has been explained to me, including the potential risks/discomforts associated with the research. I have also been given the opportunity to ask questions about the research and received satisfactory answers, and know that I may continue to ask questions and receive satisfactory answers throughout the study.

I understand that I am free to withdraw my participation in the research at any time and that if I do I will not be subjected to any penalty or discriminatory treatment. I also understand my participation in this project is on a voluntary basis, and no remuneration will be provided by Brock University in exchange for my participation. I understand that I must expectorate wine samples following tasting, and that failure to comply with this will cause me to immediately withdraw from the study.

I understand that any information or personal details gathered in the course of this research about me are confidential and that neither my name nor any other identifying information will be used or published without my written permission.

This study has been reviewed and approved by the Brock University Research Ethics Board.

I understand that if I have any complaints or concerns about this research I can contact: Research Ethics Officer, Office of Research Services, Brock University, Ph: 905 688 5550, ext: 3035; **reb@brocku.ca**

Your Name: .....

Signature:

.....

Date:.....

Please check this box if you ARE NOT interested in being contacted to participate in future sensory study performed by the Pickering lab. ☐

APPENDIX F: QUESTIONNAIRE (CHAPTERS 3, 4, 5 AND 6)

QUESTIONNAIRE

This information will remain confidential and will be used to assist in recruiting for this study and with data analysis.

GENERAL

FULL NAME (first name, last name):

_____

I AM A BROCK UNI: STUDENT STAFF MEMBER FACULTY MEMBER OTHER (please circle)

GENDER (tick one): MALE ____ FEMALE _____ AGE _____

EMAIL ADDRESS: _____

PHONE NO: DAY: _____ EVENING: _____

HEALTH (enter '**Y**' for yes or '**N**' for no unless otherwise indicated)

DO YOU HAVE ANY OF THE FOLLOWING? -

DENTURES ____

DIABETES ____

SINUS PROBLEMS ____

HYPOGLYCAEMIA ____

FOOD ALLERGIES ____ If yes, please specify

_____

ORAL DISEASE ____ If yes, please specify

_____

ARE YOU ALLERGIC TO WINE? ____ If yes, please specify

_____

DO YOU TAKE ANY MEDICATIONS WHICH AFFECT YOUR SENSES, ESPECIALLY TASTE AND SMELL ?

_____

—

WINE EXPERIENCE:

ON AVERAGE, ON HOW MANY OCCASIONS PER MONTH DO YOU DRINK WINE? _____

ARE YOU CURRENTLY EMPLOYED BY A WINERY OR COMPANY INVOLVED IN THE WINE INDUSTRY?: (Y/N) _____ PLEASE ELABORATE:

_____

Many thanks for your participation!

Nicole Gaudette

MSc. Student, Dept. of Biological Sciences, Brock University

Principle Investigator: Ms. Nicole Gaudette, MSc. Student, Dept. of Biological Sciences, Brock University, (905) 688-5550 ext: 4719, ng06qz@brocku.ca

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APPENDIX G: TRIANGLE TEST INSTRUCTIONS (CHAPTER 3)

Flavour Evaluation:

In front of your are three samples. Two of the three samples are the same, one of the samples is different. Please SMELL and TASTE the samples in the order indicated below and identify the DIFFERENT sample.

Visual Evaluation:

In front of your are three samples. Two of the three samples are the same, one of the samples is different. Please VISUALLY EXAMINE the samples in the order indicated below and identify the DIFFERENT sample.

APPENDIX H: FLAVOUR EVALUATION AND RINSING INSTRUCTIONS FOR DESCRIPTIVE ANALYSIS PANEL (CHAPTER 3)

Flavour Evaluation:

1) Please SMELL the wines in the order presented and mark the intensity of the attributes listed on the line scales.

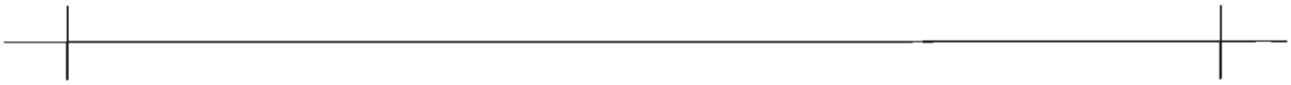
2) Please TASTE the wines in the order presented and mark the intensity of the attributes listed on the line scales.

Rinsing:

White wines: Please rinse your mouth twice with water followed by eating a cracker.

Red wines: Please rinse your mouth twice with water, then once with pectin. Follow by eating a cracker.

APPENDIX I: VISUAL ANALOG SCALE (VAS) USED FOR RATING THE INTENSITY OF OROSENSORY SENSATIONS (CHAPTER 3, 4, 5 AND 6)



absent high

Chapter 3: Descriptive analysis ratings for:

Aroma - apple, citrus, honey, ripe banana, candied banana, stonefruit, floral, vegetal

Flavour - apple, citrus, stonefruit, vegetal, honey, salty, acidity, astringency, bitterness, heat

Chapter 4:

Sensations: sweet, salty, sour, savory, bitter, astringent, 'other'

Chapter 5 and 6:

Sensations: sweet, bitter, astringent, in-mouth aroma, 'other'

APPENDIX J: INTENSITY RATINGS FOR ADDITIONAL TASTES AND 'OTHER' (CHAPTER 4)

Table A2. Perceived intensity ratings for sweet, savory, salty, sour and 'other' from solutions containing high and low concentrations of (+)-catechin or caffeine paired with high and low concentrations of magnesium sulfate, zinc sulfate, homoeridictyol sodium salt, carboxymethylcellulose, or β-cyclodextrin. Data represent mean values of duplicate reps ± SEM. Means sharing the same letter do not differ significantly within each sensation (Tukey's $HSD_{0.05}$).

Stimuli	Sweet	Savory	Salty	Sour	Other
Low (+)-catechin (LCat)	0.50 a ± 0.34	0.20 b ± 0.11	0.40 a ± 0.19	0.20 a ± 0.12	0.10 c ± 0.11
LCat-high Mg^{+2}	0.90 a ± 0.35	0.10 b ± 0.27	0.00 a ± 0.00	0.10 a ± 0.10	0.30 bc ± 0.27
LCat-low Mg^{+2}	1.1 a ± 0.64	0.10 b ± 0.40	0.00 a ± 0.00	0.40 a ± 0.43	0.60 bc ± 0.40
LCat-high Zn^{+2}	0.20 a ± 0.15	3.0 a ± 0.97	0.10 a ± 0.10	0.00 a ± 0.01	3.4 a ± 0.97
LCat-low Zn^{+2}	0.40 a ± 0.30	1.9 ab ± 0.63	0.40 a ± 0.22	0.10 a ± 0.10	1.4 b ± 0.63
LCat-high HED	1.4 a ± 0.72	0.70 b ± 0.15	0.10 a ± 0.10	0.00 a ± 0.00	0.30 bc ± 0.15
LCat-low HED	1.2 a ± 0.68	0.20 b ± 0.20	0.10 a ± 0.11	0.20 a ± 0.21	0.40 bc ± 0.20
LCat-high CMC	0.70 a ± 0.57	1.7 ab ± 0.72	0.20 a ± 0.19	0.20 a ± 0.15	1.1 bc ± 0.72
LCat-low CMC	0.70 a ± 0.37	0.50 b ± 0.46	0.20 a ± 0.13	0.00 a ± 0.01	0.70 bc ± 0.46
LCat-high CYCLO	1.3 a ± 0.58	0.00 b ± 0.57	0.30 a ± 0.24	0.10 a ± 0.12	0.70 bc ± 0.57
LCat-low CYCLO	0.60 a ± 0.34	0.10 b ± 0.03	0.00 a ± 0.00	0.10 a ± 0.10	0.00 c ± 0.03
High (+)-catechin (HCat)	0.60 a ± 0.35	0.30 a ± 0.18	0.10 bc ± 0.07	0.40 a ± 0.21	0.30 a ± 0.18
HCat-high Mg^{+2}	1.3 a ± 0.54	0.40 a ± 0.24	0.00 c ± 0.01	0.20 a ± 0.15	0.40 a ± 0.24
HCat-low Mg^{+2}	1.2 a ± 0.45	0.50 a ± 0.25	0.20 bc ± 0.15	0.20 a ± 0.20	0.50 a ± 0.25
HCat-high Zn^{+2}	0.20 a ± 0.09	1.3 a ± 0.72	0.10 bc ± 0.08	0.20 a ± 0.16	1.6 a ± 0.72
HCat-low Zn^{+2}	0.20 a ± 0.14	0.40 a ± 0.24	0.20 bc ± 0.12	0.40 a ± 0.36	0.40 a ± 0.24
HCat-high HED	1.7 a ± 0.99	0.70 a ± 0.34	0.50 b ± 0.25	0.10 a ± 0.10	0.70 a ± 0.33
HCat-low HED	1.6 a ± 0.84	1.1 a ± 0.78	0.00 c ± 0.00	0.20 a ± 0.21	1.1 a ± 0.78
HCat-high CMC	0.70 a ± 0.41	1.3 a ± 0.92	1.0 a ± 0.61	0.30 a ± 0.17	1.3 a ± 0.92
HCat-low CMC	0.80 a ± 0.46	0.90 a ± 0.60	1.2 a ± 0.53	0.10 a ± 0.10	0.90 a ± 0.60
HCat-high CYCLO	1.1 a ± 0.52	0.30 a ± 0.19	0.20 bc ± 0.23	0.10 a ± 0.10	0.30 a ± 0.19

HCat-low CYCLO	1.1 a ± 0.69	0.20 a ± 0.15	0.00 c ± 0.00	0.00 a ± 0.00	0.20 a ± 0.15
Low caffeine (LCaff)	0.60 a ± 0.31	0.20 b ± 0.20	0.50 a ± 0.22	0.10 a ± 0.07	0.20 b ± 0.20
LCaff -high Mg^{+2}	1.3 a ± 0.63	0.10 b ± 0.	0.00 a ± 0.04	0.10 a ± 0.08	0.10 b ± 0.08
LCaff -low Mg^{+2}	0.60 a ± 0.38	0.10 b ± 0.052	0.00 a ± 0.00	0.10 a ± 0.06	0.10 b ± 0.05
LCaff -high Zn^{+2}	0.80 a ± 0.46	3.0 a ± 1.2	0.80 a ± 0.48	0.40 a ± 0.26	3.0 a ± 1.2
LCaff -low Zn^{+2}	0.50 a ± 0.47	1.9 ab ± 0.60	0.00 a ± 0.00	0.10 a ± 0.08	1.9 ab ± 0.60
LCaff -high HED	0.60 a ± 0.29	0.70 b ± 0.40	0.50 a ± 0.46	0.00 a ± 0.00	0.70 b ± 0.40
LCaff -low HED	1.1 a ± 0.48	0.20 b ± 0.15	0.00 a ± 0.00	0.20 a ± 0.14	0.10 b ± 0.15
LCaff -high CMC	0.70 a ± 0.33	1.7 ab ± 0.81	0.10 a ± 0.10	0.00 a ± 0.00	1.7 ab ± 0.81
LCaff -low CMC	1.0 a ± 0.55	0.50 b ± 0.50	0.20 a ± 0.14	0.30 a ± 0.25	0.50 b ± 0.50
LCaff -high CYCLO	2.3 a ± 1.1	0.00 b ± 0.04	0.20 a ± 0.19	0.00 a ± 0.02	0.00 b ± 0.04
LCaff -low CYCLO	1.0 a ± 0.46	0.10 b ± 0.08	0.30 a ± 0.12	0.20 a ± 0.08	0.10 b ± 0.08
High caffeine (HCaff)	0.05 a ± 0.05	0.50 b ± 0.28	0.30 a ± 0.25	0.00 b ± 0.00	0.50 b ± 0.28
HCaff -high Mg^{+2}	1.1 a ± 0.57	0.10 b ± 0.05	0.20 a ± 0.15	0.00 b ± 0.00	0.10 b ± 0.05
HCaff -low Mg^{+2}	0.10 a ± 0.08	0.20 b ± 0.15	0.20 a ± 0.16	0.70 a ± 0.50	0.20 b ± 0.15
HCaff -high Zn^{+2}	1.0 a ± 0.82	2.3 a ± 0.87	0.50 a ± 0.49	0.30 b ± 0.25	2.3 a ± 0.87
HCaff -low Zn^{+2}	0.50 a ± 0.40	0.40 b ± 0.18	0.20 a ± 0.11	0.30 b ± 0.19	0.40 b ± 0.18
HCaff -high HED	0.90 a ± 0.68	0.30 b ± 0.13	0.00 a ± 0.00	0.00 b ± 0.00	0.30 b ± 0.13
HCaff -low HED	0.70 a ± 0.66	0.50 b ± 0.37	0.20 a ± 0.17	0.00 b ± 0.05	0.50 b ± 0.37
HCaff -high CMC	0.20 a ± 0.11	1.0 ab ± 0.63	0.80 a ± 0.51	0.20 b ± 0.20	1.0 ab ± 0.63
HCaff -low CMC	1.3 a ± 0.77	0.00 b ± 0.01	0.50 a ± 0.25	0.00 b ± 0.00	0.00 b ± 0.01
HCaff -high CYCLO	0.40 a ± 0.19	0.50 b ± 0.46	0.70 a ± 0.40	0.30 b ± 0.30	0.50 b ± 0.46
HCaff -low CYCLO	0.10 a ± 0.12	0.00 b ± 0.00	0.30 a ± 0.22	0.00 b ± 0.00	0.00 b ± 0.00

APPENDIX K: TASTING AND RINSING PROTOCOL (CHAPTER 4)

1) Place the nose clips firmly on your nose.

2) Pick up the glass and put the full amount of solution into your mouth.

3) Swirl the solution in your mouth for 5 seconds.

4) Spit out the sample.

5) Wait at least 10 seconds for the sensation(s) to begin to diminish (wait longer if the intensity is still increasing).

6) Rate the ***highest intensity*** you experience of all tastes, astringency, and/or other.

7) Remove the nose clips when you are finished evaluating the sample.

NOTE: You may not perceive all of the sensations listed. That is normal. If you do not perceive a sensation, mark it at 'absent.'

APPENDIX L: TASTING AND RINSING PROTOCOL (CHAPTER 5 AND 6)

1) Pick up the glass, remove the lid, and put the full amount of solution into your mouth.

2) Replace the lid onto the glass.

3) Swirl the solution in your mouth for 5 seconds.

4) Spit out the sample.

5) Wait at least 10 seconds for the sensation(s) to begin to diminish (wait longer if the intensity is still increasing).

6) Rate the ***highest intensity*** you experience of all the tastes, astringency, aroma, and 'other.'

7) Please describe 'other' using the space provided on the following screen.

NOTE: You may not perceive all of the sensations listed. That is normal. If you do not perceive a sensation, mark it as 'absent.'

APPENDIX M: pH VALUES FOR TREATMENTS (CHAPTER 5 AND 6)

Table A3. pH values for all binary, ternary and quaternary solutions, in duplicate measurement (± SD) presented in Chapter 5 and 6.

Treatment - binary	**pH**	**Treatment - ternary**	**pH**	**Treatment - quaternary**	**pH**
CAT	6.04 ± 0.01	CAT + SUC + HD	6.96 ± 0.00	CAT + SUC + CD + V	5.09 ± 0.00
CAT + SUC	6.07 ± 0.00	CAT + SUC + CD	5.01 ± 0.01	CAT + SUC + CD + T	5.19 ± 0.00
CAT + REB	6.09 ± 0.00	CAT + SUC + V	6.02 ± 0.01	CAT + SUC + HD + V	6.74 ± 0.00
CAT + HD	6.93 ± 0.02	CAT + SUC + T	6.17 ± 0.01	CAT + SUC + HD + T	7.02 ± 0.00
CAT + CD	4.88 ± 0.01	CAT + REB + HD	6.91 ± 0.00	CAT + REB + CD + V	5.18 ± 0.00
CAT + V	5.93 ± 0.00	CAT + REB + CD	5.14 ± 0.00	CAT + REB + CD + T	5.21 ± 0.00
CAT + T	6.16 ± 0.00	CAT + REB + V	5.88 ± 0.02	CAT + REB + HD + V	6.63 ± 0.00
		CAT + REB + T	6.14 ± 0.01	CAT + REB + HD + T	6.98 ± 0.00
		CAT + CD + V	5.35 ± 0.00		
		CAT + CD + T	5.15 ± 0.00		
		CAT + HD + V	6.96 ± 0.00		
		CAT + HD + V	6.72 ± 0.01		